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Ameliorative Effect of Vitamin E on Chemotherapy Induced Side Effects in Rat Liver

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ABSTRACT

The aim of the study was to evaluate the extent of change in activities of antioxidant enzymes and lipid peroxidation caused by CPT and protective efficacy of Vitamin E over detrimental effects induced by CPT in male Wistar rat. Male Wistar rats 120±20 g were categorized in four groups. Two groups of rats were administered with CPT (6 mg kg^{-1} intravenously for 4 days); one of these groups received Vitamin E (oral dose of 6 mg kg^{-1} for 30 days) prior to CPT injections. A vehicle treated control group and a Vitamin E control group were also included. CPT treated rats showed significant increase in lipid peroxidation and glycogen levels when compared to control rats. In rats treated with CPT, abnormal changes in activities of enzymic (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase) and non-enzymic (reduced glutathione and Vitamin E) antioxidants were observed. Liver of CPT treated rats showed significant decrease in diagnostic marker enzymes such as aspartate aminotransferase and alanine aminotransferase accompanied with significant increase in alkaline phosphatase and lactate dehydrogenase levels. Pretreatment with Vitamin E reduced the oxidative stress and change in activity of antioxidant enzymes induced by CPT, thereby demonstrating the protection rendered by Vitamin E. Result of the present study suggests that Vitamin E administrated orally protected against oxidation in our experimental models and could be one approach to reducing the risk of CPT-induced side effects in clinical settings.

Key words: Camptothecin, antioxidant enzymes, lipid peroxidation, oxidative stress, α-tocopherol

INTRODUCTION

Chemotherapy has long been a cornerstone of cancer therapy. Although extensive research is done on the development of more effective and less toxic antineoplastic agents, much less attention is given to the factors that may enhance the effectiveness of existing drugs. The use of chemotherapeutic agents in cancer treatment is often accompanied by side effects caused by the occurrence of oxidative stress. Increased lipid peroxidation, reduced antioxidant vitamins, free radical trapping capacity in plasma and a marked reduction of tissue Glutathione (GSH) levels are frequently reported during chemotherapy (Goncalves *et al*., 2009). The enhanced production of Reactive Oxygen Species (ROS) damages normal tissues and therefore results in toxic side effects of chemotherapeutic agents. In particular, tissue and cells with a high proliferation rate are mostly affected by the oxidative stress (Conklin, 2000). ROS generated during cancer chemotherapy may

also decrease the efficacy of the treatment by interfering with drug induced apoptosis and cell cycle progression which are important for chemotherapeutic agents to exert their optimal effect on cancer cells (Conklin, 2004; Wessner *et al*., 2007).

Camptothecin (CPT) is a natural alkaloid extracted from the leaves and fruit of *Camptothecin acuminate* of Nyssaceae family. It is an inhibitor of DNA synthesis and a potent drug with a broad spectrum antitumor activity (Berrada *et al*., 2005). Topoisomerase I is the target of camptothecin and its chemotherapeutic derivatives. These anticancer drugs commonly referred to as "Top1 inhibitor", specifically bind at the Top1-DNA interface and forms ternary cleavable complex, thus preventing DNA religation step. Stabilization of Top1- cleavage complex by Top1 inhibitors arrests the replication fork which generates bulky DNA lesions. This leads to initiation of cellular response that includes apoptosis, DNA repair and cell cycle arrest (Pizzolato and Saltz, 2003; Sordet *et al*., 2004).

The major drawbacks of using camptothecin, as an anticancer drug include water insolubility, lactone instability, reversibility of the drug-target interaction and drug resistance. Toxic effects include myelosuppression, gastrointestinal toxicity, fever, diarrhoea, hemorrhagic cystitis, nausea, vomiting, alopecia, reversible leucopenia and thrombocytopenia (Mross *et al*., 2004; Boige *et al*., 2000). Camptothecin and its analogue are known to induce apoptosis in uterine squamous cell carcinoma cell line SiHa (Ha *et al*., 2009) and ROS formation in a promyelocytic leukemia HL-60 and MCF-7 cell lines (Timur *et al*., 2005). H_2O_2 generation is also reported in the process of camptothecin mediated mitochondrial dysfunction leading to programmed cell death (Sen *et al*., 2004). DNA double strand breaks are produced during DNA synthesis in presence of CPT proposing that it should not be toxic to non-dividing cells but on contrary CPT induces significant dose-dependent cell death of primary cerebellar granule neurons *in vitro* (Uday Bhanu and Kondapi, 2010).

Vitamin E, a free-radical scavenger in the lipid compartment of cells and serum is known for its beneficial antioxidant effects for number of chronic diseases, including cancer (Kline *et al*., 2004). Increased serum Vitamin E levels have been reported to decrease lipid peroxidation, inhibit protein kinase C, 5-lipoxygenase, smooth muscle cell proliferation, platelet aggregation and the oxygen burst in neutrophils (Brigelius-Flohe *et al*., 2002; Peralta *et al*., 2006). Pretreatment with Vitamin E supplementation has been proven to show neuroprotective effect (Pace *et al*., 2003) and has been reported to prevent many changes in serum enzymes and protect increase in hematocrit, fall in leukocyte count, hemoglobin level, mean osmotic fragility of erythrocytes (Nayma *et al*., 2006). The present study was designed to understand antioxidant potential of Vitamin E on camptothecin induced oxidative stress.

MATERIALS AND METHODS

Drugs and chemicals: Camptothecin, α- tocopherol acetate, glutathione reductase, epinephrine and reduced glutathione was purchased from Sigma Chemicals, St Louis, MO, USA. All other chemicals used were of analytical grade and solvents were of Qualigen grade.

Animal model: Adult male albino rats of Wistar strain (120±20 g) were obtained in the month of January 2009 from Bharat Serum Pvt. Ltd, Thane, Navi Mumbai, India. The animals were maintained under standard conditions of humidity, temperature (25±2°C) and light (12 h light/dark). They were fed standard rat pelleted diet obtained from Lipolin India and water *ad libitum*. Experimental animals were handled according to the Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design: Following the acclimatization period, the rats were randomly divided into 4 groups consisting of six animals each.

Group I: Control Rats were given saline solution (0.9% NaCl) for four consecutive days, intravenously

Group II: Rats were injected CPT (6 mg kg^{-1} body weight) dissolved in Dimethyl Sulphoxide (DMSO) for four consecutive days, intravenously

Group III: Rats served as control group for Vitamin E and received α-tocopherol (6 mg kg^{-1} body weight) orally daily for a period of 30 days

Group IV: Rats received α-tocopherol prior to CPT injection as described for group 2 and 3 rats

At the end of the experimental period the animals were killed by decapitation. Liver was excised immediately, washed with ice-cold saline and processed for following analysis.

Biochemical analysis: The liver tissue was homogenized with the help of a glass-Teflon homogenizer in 0.01 M Tris-HCl buffer (pH-7.4) for enzyme assays and in 0.15 M KCl for lipid peroxidation studies. The aliquots of the homogenate were suitably processed for the assessment of following biochemical parameters.

Lipid peroxidation: Lipid peroxidation was determined by the procedure of Ohkawa *et al.* (1979). Malondialdehyde (MDA), formed as an end product of lipid peroxidation, served as a measure of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm. Tetramethoxy propane was used as standard.

Estimation of antioxidant enzymes: Superoxide Dismutase (SOD) was assayed according to the method of Misra and Fridovich *et al.* (1972). The method is based on the ability of superoxide dismutase to inhibit auto-oxidation of adrenaline to adrenochrome at alkaline pH. The unit of enzyme activity is defined as the enzyme required for 50% inhibition of adrenaline auto-oxidation. Catalase activity was measured using the method of Clairborne (1985) with hydrogen peroxide as a substrate. The method relies on decomposition of hydrogen peroxide which is indicated by a decrease in absorbance at 240 nm. Glutathione-S-transferase (GST) activity was estimated by Habig *et al.* (1974) method based on enzyme-catalysed condensation of glutathione with the model substrate, 1-choloro, 2,4-dinitrobenzene. The product formed (2,4-dinitrophenyl-glutathione) absorbs light at 340 nm which facilitated the analysis of enzyme activity based on product formation. Glutathione Peroxidase (GPX) was assayed by the method of Paglia and Valentine (1967) which depends on the oxidation of NADPH at 340 nm using hydrogen peroxide. Glutathione Reductase (GRD) activity was measured by the method of Carlberg and Mannervik (1975). In presence of glutathione reductase, hydrogen is transferred from NADPH to GSSG and reaction is monitored at 340 nm spectrophotometrically.

Assessment of non-enzymic antioxidant: Total Reduced Glutathione (GSH) was estimated by the method of Moron *et al.* (1979), where the colour developed was read at 412 nm. Tocopherol (Vitamin E) was assayed by the method of Bieri *et al.* (1964).

Estimation of diagnostic marker enzymes: The aminotransferases are most frequent indicator of hepatotoxicity. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) both

were estimated by King (1965) method with a difference that former uses DL-Aspartate and later DL-Alanine as a substrate. Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) were also assayed by King method (King, 1965; King *et al*., 1951). Protein content was determined by the method of Lowry *et al*. (1951).

Estimation of glycogen: Tissue glycogen was extracted and estimated by the method of Morales *et al*. (1973). Alkali extract of the tissue was prepared and mixed with ammonium acetate to precipitate glycogen. After extraction, glycogen was hydrolyzed to glucose using acid where glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with absorption maximum at 630 nm.

Data analysis: The results were expressed as Mean±Standard Deviation (SD) for six animals in each group. Statistical significance of biochemical assays has been analyzed by Student's t-test and given respective symbols in the tables.

RESULTS

There were no distinctive clinical signs, mortality or morbidity observed in any of the experimental groups during the study period. Treatment of rat with camptothecin causes no significant change in body or tissue weight.

Effect of camptothecin and Vitamin E on lipid peroxidation in rats liver is shown in Fig. 1. Lipid peroxide status reveals significant ($p<0.001$) increase in group II which highlights CPT induced oxidative damage. However Vitamin E prevented CPT induced alterations significantly ($p<0.001$) in-group IV. The CPT induced group shows a significant depletion in GSH ($p<0.01$). Vitamin E pretreatment brought about a significant increase ($p<0.05$) in the level of these antioxidants as against group II (Fig. 2). Hepatic tissue of CPT treated rats (Group II) showed 1.92 folds depletion in Vitamin E when compared to control (Fig. 3) whereas in Group III and Group IV showed 1.58 and 1.49 fold rise in Vitamin E level as compared to group I. A significant increase ($p<0.001$) in

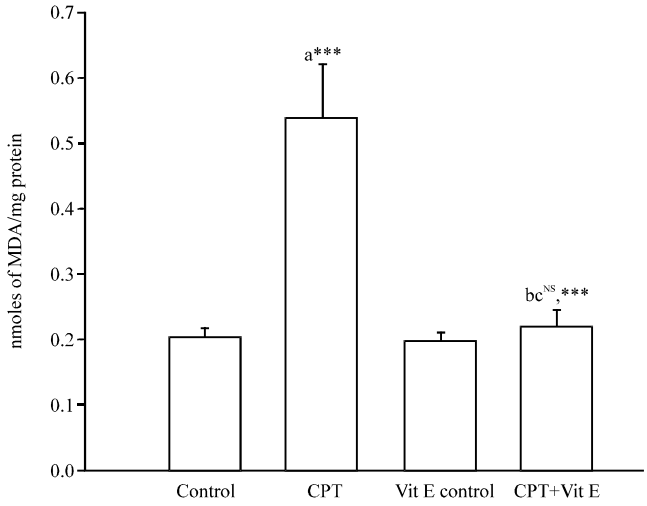

Fig. 1: Effect of camptothecin and Vitamin E on lipid peroxidation in rats liver. Values are expressed as Mean±SD for six rats in a group. Comparisons are made between (a) Group I and II (b) Group I and IV (c) Group II and IV. ***$p<0.001$ NS: Non significant

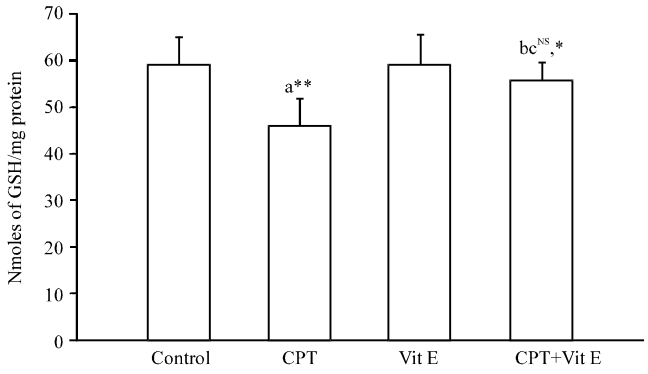

Fig. 2: Effect of camptothecin and Vitamin E on the levels of GSH in rat liver. Values are expressed as Mean±SD for six rats in a group. Comparisons are made between (a) Group I and II (b) Group I and IV (c) Group II and IV. *p<0.05, **p<0.01 NS: Non significant

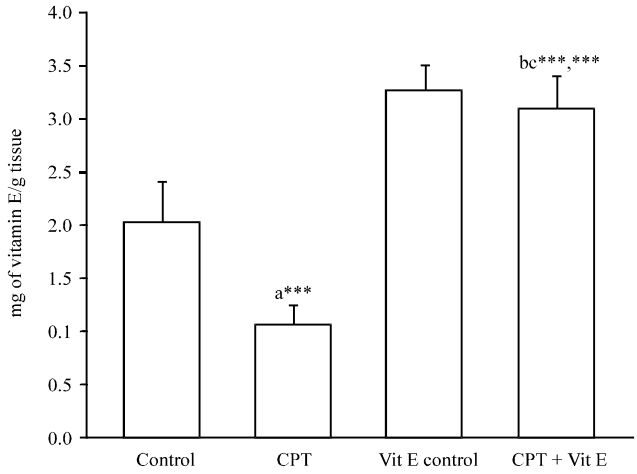

Fig. 3: Change in liver Vitamin E level in all groups after camptothecin and Vitamin E administration. Values are expressed as Mean±SD for six rats in a group. Comparisons are made between (a) Group I and II (b) Group I and IV (c) Group II and IV. ***p<0.001

hepatic glycogen content (Fig. 4) is noted in CPT treated animals when compared to control whereas Vitamin E administration causes significant (p<0.001) reversal of elevated level to normal glycogen content when compared to group II.

Administration of CPT causes a significant change in antioxidant enzymes. Table 1 presents the altered activity of antioxidant enzymes SOD, Catalase, GPX, GST and GRD in the liver of CPT treated groups and protection rendered by Vitamin E. When compared to control, CPT injected rats showed increased activities of SOD, Catalase, GPX and GST by 66.89, 62.12, 48.79 and 51.87%, respectively and decreased activity of GRD by 22.18%. Treatment of group IV with Vitamin E, significantly reversed CPT induced alteration in the levels of SOD, Catalase, GPX, GST and GRD. The restoration of enzymic and non-enzymic antioxidant levels towards control range by Vitamin E indicates its protective effect against oxidative stress induced changes in antioxidant molecules.

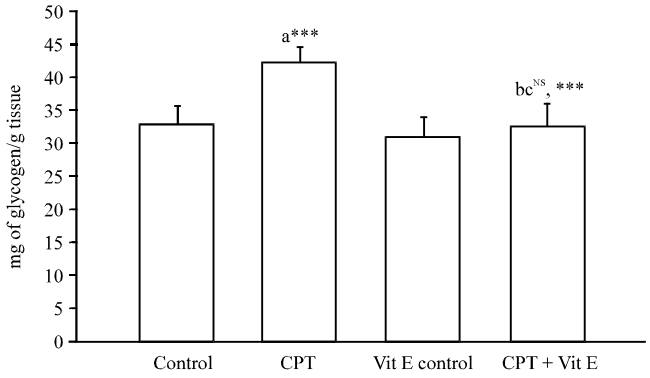

Fig. 4: Effect of camptothecin and Vitamin E on liver glycogen. Values are expressed as Mean±SD for six rats in a group. Comparisons are made between (a) Group I and II (b) Group I and IV (c) Group II and IV. ***p<0.001 NS: Non significant

Table 1: Effect of Camptothecin and Vitamin E on the activities of antioxidant enzymes in rat liver

Antioxidant enzymes	Group I (Control)	Group II (CPT)	Group III (Vitamin E)	Group IV (Vitamin E+CPT)
SOD	0.101±0.01	0.168±0.03a**	0.098±0.01	0.114±0.02bc NS,*
Catalase	1.270±0.22	2.059±0.40a**	1.302±0.19	1.362±0.27bcNS,**
GPX	0.469±0.07	0.698±0.11a**	0.459±0.06	0.511±0.10 bcNS,*
GST	1.342±0.19	2.038±0.29a***	1.378±0.20	1.467±0.24bc NS,**
GRD	0.188±0.02	0.146±0.01a**	0.181±0.03	0.174±0.02bcNS,*

Values are expressed as Mean ± SD for six rats in each group. Enzyme activities are expressed as follows: SOD, superoxide dismutase: units/mg protein (1U = amount of enzyme that inhibits the auto-oxidation of Epinephrine by 50%); CAT, catalase: µmol of H_2O_2 consumed/min/mg protein; GPX, glutathione peroxidase: nmoles of NADPH oxidized/ min/ mg protein; GST, glutathione-S-transferase: micromoles of CDNB conjugated / min/ mg protein; GRD, glutathione reductase: nmoles of NADPH oxidized/min/mg protein; Comparisons are made between: 'a' Groups I–II; 'b' Groups I and IV; 'c' Group II and IV, Statistical significance:*p<0.05, **p<0.01, ***p<0.001

Table 2: Effect of Camptothecin and Vitamin E on diagnostic marker enzymes

Marker enzymes	Group I (Control)	Group II (CPT)	Group III (Vitamin E)	Group IV (Vitamin E+CPT)
AST	1.65±0.31	0.77±0.16a***	1.67±0.32	1.41±0.22bcNS,***
ALT	1.13±0.22	0.63±0.10a***	1.13±0.18	1.11±0.21bcNS,***
ALP	5.50±1.03	9.70±1.92 a***	5.98±0.99	5.39±1.0 bcNS,***
LDH	7.40±0.60	10.67±2.02 a**	7.30±0.95	7.66±1.01 bcNS,**

Values are expressed as Mean±SD for six rats in each group. Enzyme units are expressed as follows: AST, ALT, LDH: nmoles of pyruvate liberated/ min/ mg protein; ALP: nmoles of phosphorus liberated/ min/ mg protein; Comparisons are made between: 'a' Groups I–II; 'b' Groups I and IV; 'c' Group II and IV Statistical significance: *p<0.05, **p<0.01, ***p<0.001

Change in levels of AST, ALT, alkaline phosphatase and lactate dehydrogenase that are hallmark of hepatocellular damage in CPT treated rat liver and associated modulatory effect of Vitamin E is shown in Table 2. In group II, CPT induced significant (p<0.001) decrease in levels of AST and ALT and increase in alkaline phosphatase. In group IV, these drug induced alteration was absent and enzyme levels were resumed to normal indicating ameliorative nature of Vitamin E. When compared with control, LDH increased significantly (p<0.01) in group II, whereas group IV showed significant decrease (p<0.01) in level of LDH against group II. The normal rats receiving Vitamin E alone (Group III) did not show any significant change when compared with control rats (Group I), indicating that it does not per se have any adverse effects.

DISCUSSION

Most of the major classes of antineoplastic agents have well-established mechanism of action which does not rely on generation of free radical intermediates. These include the antifolates, nucleotide and nucleoside analogs that interfere with DNA synthesis, vinca alkaloid that hinders microtubule formation, epipodophyllotoxins that inhibit DNA topoisomerase II and CPT that interferes with topoisomerase I activity. CPT is known to generate a high level of oxidative stress in normal biological system which could be the reason of CPT induced side effects (Conklin, 2004). Gorman *et al.* (1997) has reported increased ROS formation in a promyelocytic leukemia HL-60 cell line after camptothecin and SN-38 (analog of CPT) treatment.

Lipid peroxidation has been postulated to precede through a complex process in biological membrane system i.e., it involves a destruction and rearrangement of the double bonds in unsaturated lipids by propagation of lipid free radical. The accumulation of lipid peroxides can introduce hydrophilic moieties into the membrane hydrophobic phase and thus can result in alteration of membrane permeability and cell function (Geetha *et al.*, 1990). The present data reveal that CPT administration produced a marked oxidative impact as evidenced by the significant increase in lipid peroxides. The increase in lipid peroxides might result from increased production of free radicals. This finding is in agreement with other investigation that reported CPT as a strong inducer of oxidative stress and free radical detoxifying enzymes (Timur *et al.*, 2005).

Depletion of tissue GSH is prime factor which can impair the cell's defense against the ROS toxicity and may lead to peroxidative cell injury as it is required for many critical cell processes. It plays a crucial role in maintenance and regulation of thiol-redox status of the cell (Ballatori *et al.*, 2009; Husain *et al.*, 2004). GSH serves as both a nucleophile and an effective reductant by interacting with many oxidizing and electrophilic compounds. It acts as non-enzymic antioxidant by direct interaction of SH group with ROS or can participate in detoxification reaction of ROS, as a co-enzyme (Selvakumar *et al.*, 2006). In the present study level of Glutathione (GSH) was decreased significantly ($p<0.01$) in liver. But the level of GSH in Vitamin E administered rats was restored to near normal. The GSH level is maintained either by its synthesis by the enzyme gamma-glutamylcysteine synthase or by the conversion of GSSG to GSH in the presence of NADPH dependent glutathione reductase (Li *et al.*, 1989). Since GSH would be utilized by free radical detoxifying enzymes, it is expected to increase the GSSG content of the cell. In order to maintain the redox potential of the cell the GSSG formed should be converted to GSH by Glutathione Reductase (GRD). Decrease in GRD levels can be the reason for decrease in the GSH found in the present study. Another reason proposed for depletion of GSH content may be attributed to the direct conjugation of drug and its metabolites with free or protein bound SH groups (Yuan *et al.*, 1991), thereby interfering with the antioxidant functions. Shanmugarajan *et al.* (2008) reported similar result of drug induced GSH depletion and generation of ROS in hepatic tissue as a sign of oxidative injury. Timur *et al.* (2005) supported our finding of decrease in levels of GSH in MCF-7 human breast cancer cell line due to cytotoxic effect of water-soluble derivative of CPT.

Vitamin E is the main lipid soluble chain breaking antioxidant which protects unsaturated fatty acids from peroxidation. The decrease in Vitamin E clearly indicates overproduction of ROS and other free radicals in CPT treated animals whereas level of Vitamin E in group III and IV increases more than control because of 30 days of Vitamin E treatment. When compared, group IV illustrates decreased amount of Vitamin E than group III that signify the involvement of Vitamin E in detoxification of reactive oxygen species and free radical intermediates.

Glycogen content of liver showed significant increase in camptothecin treated animals which could be attributed to a reduction in the activity of glycogen phosphorylase and glucose-6-phosphatase (Geetha *et al.*, 1989). As the lipid peroxidation products are reported to deactivate enzymes (Kim *et al.*, 1985), the alteration induced in glycogen content might have been through the lipid peroxidative nature of CPT. Pretreatment of Vitamin E prevents this alteration significantly, because of the antioxidant nature of α-tocopherol against lipid peroxidation induced by CPT (Doroshow, 1983).

The present study shows changes in anti-peroxidative enzyme profile. Catalase, GPX and GST are class of antioxidant enzymes responsible for scavenging hydrogen peroxide and organic hydroperoxides. The present investigation shows significant increase in GPX, Catalase ($p<0.01$) and GST ($p<0.001$) when compared to control group. These peroxides cause increased rate of lipid peroxidation (Saito and Takahashi, 2000) which justifies inducible synthesis of Catalase and GPX (Choi *et al.*, 2009) as an adaptive mechanism to detoxify the reactive species in order to minimize tissue damage. The activity of GRD, the enzyme crucial for regeneration of reduced glutathione was significantly ($p<0.01$) less than that of control. This enzyme contains one or more sulphydryl group residues which are essential for catalytic activity and are vulnerable to free radical mediated inactivation (Gutierrez-Correa and Stoppani, 1997). The reduced GRD activity could be correlated to enzyme inactivation by ROS. This can be justified by the fact that under high oxidative stress GSSG can be effluxed out of the cell (Muller, 1996) or they form mixed disulfides with the cellular proteins (Brigelius, 1985) leading to alteration of their function. Superoxide Dismutase (SOD) has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical (Curtis *et al.*, 1972). In present study, SOD shows significant ($p<0.01$) increase of 66.89% in CPT treated group.

In the assessment of liver damage caused by CPT, the determination of enzyme levels such as AST and ALT was carried out. Necrosis or membrane damage releases the enzyme into circulation and reduces the levels of these enzymes in liver. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Dhir and Dhand, 2010). Administration of camptothecin caused a significant ($p<0.001$) decline in AST and ALT enzyme levels in liver when compared to control which can be due to cellular leakage. There was a significant ($p<0.001$) restoration of these enzyme levels on administration of Vitamin E. The reversal of decreased enzymes in CPT-induced liver damage by Vitamin E may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that changed levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Nakbi *et al.*, 2010). ALP is a membrane bound enzyme located in bile canalicular pole of hepatocyte. During oxidative stress, this enzyme level was elevated, as a response to drug therapy (Sudhakar *et al.*, 2010). LDH catalyzes the reversible oxidation of lactate to pyruvate in the last step of glycolysis. In the present study, LDH activity was increased in liver of rats exposed to CPT when compared to control rats (Table 2). The LDH activity increases during conditions favoring anaerobic respiration to meet the energy demands, when aerobic condition is decreased. Elevation in the activity of LDH indicates reduced TCA cycle activities (Reddy *et al.*, 2010).

In this study, pretreatment with an antioxidant significantly reversed the upsurge in the lipid peroxide levels and the altered antioxidant enzymes status. Similar to our finding Selvakumar and colleagues have reported that use of lipoic acid can ameliorate tissue peroxidative damage and

abnormal antioxidant levels in drug induced hepatotoxicity (Selvakumar *et al.*, 2005). It is confirmed that Vitamin E blocks lipid peroxidation-mediated toxicity while not imparing the antitumor property of the drug (Lamson and Brignall, 1999). Vitamin E is an important antioxidant found in the plasma and tissue. Vitamin E is a fat-soluble molecule present in blood lipoprotein and tissue to detoxify the free radicals. α-tocopherol quenches and reacts with singlet oxygen and OH^* radical but the major antioxidant action, in biological membranes, under most condition is to react with lipid peroxy and alkoxy radicals donating labile hydrogen to them and so terminating the chain reaction of peroxidation by scavenging chain propagating radicals (Halliwell and Gutteridge, 1989). If generation of free oxidative radicals by a drug or a free radical intermediate of the chemotherapeutic agent plays a role in its cytotoxicity, antioxidant may interfere with the drug's neoplastic activity. However, if the reactive species and free radical intermediate are responsible only for the drug's side effects, antioxidants may actually reduce the severity of such adverse effects without interfering with the drug's antineoplastic activity (Conklin, 2004).

CONCLUSION

From these observations it is possible to conclude that Vitamin E may reduce oxidative stress in CPT-induced hepatotoxicity by mitigation of lipid peroxides through scavenging of free radicals and by restoring the activities of antioxidant enzyme near normal range. Vitamin E also restores the change in hepatic glycogen content significantly by scavenging reactive species which has been theorized to inactivate the enzyme involved in glycogen degradation. Further studies are warranted in order to understand how CPT blocks glycogenolysis along with role and mechanism of this readily available dietary supplement in CPT toxicity.

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