ACUTE TOXICITY OF CAMPTOTHECIN AND INFLUENCE OF 
α-TOCOPHEROL ON HEMATOLOGICAL AND 
BIOCHEMICAL PARAMETERS

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Abstract: Camptothecin (CPT), a natural alkaloid from Camptothecin acuminata is a potent 
drug with a broad spectrum antitumor activity. Its use has been limited due to wide ranging 
toxicities and oxidative stress that have been implicated as side effects. The present study is an 
attempt of modulating CPT induced toxicities by using vitamin E as a nutritional supplement. 
Hematological parameters such as RBC, WBC and platelet counts that decreased after 
camptothecin injection were found to be restored in rats which were pretreated with 
α-tocopherol. Enhanced levels of lipid peroxides in animals administered with CPT showed significant revision 
after vitamin E administration. Improvement in the alterations of other chemical constituents 
in blood like glucose, urea, uric acid, hemoglobin, cholesterol and triglycerides were also 
observed in animals pretreated with vitamin E suggesting a prophylactic role of vitamin E in 
CPT mediated side effects.

Key words: Camptothecin toxicity, α-tocopherol, Lipid peroxidation.

INTRODUCTION

The use of chemotherapeutic agents in the treatment 
of cancer is hampered and complicated by toxic side 
effects. Many types of chemotherapy destroy cancer 
cells by generating free radicals, which can cause 
cellular damage. Unfortunately, these free radicals 
are not discriminatory in their destructive action, 
leading to undesirable side effects and sometimes 
even new cancers. Enhanced lipid peroxidation, 
reduction of antioxidant vitamins, free radical trapping 
capacity in plasma, and a marked reduction of tissue 
glutathione (GSH) levels are frequently detected 
during chemotherapy (1-3).

Camptothecin (CPT) is a cytotoxic plant alkaloid 
extracted from the leaves and fruit of Camptothecin 
acuminata of Nyssaceae family. It is an inhibitor of 
DNA synthesis and active against breast, ovarian, 
gastric and colorectal cancers [4]. Topoisomerase I 
is the target of camptothecin and its chemotherapeutic 
derivatives. Though CPT has shown promises in 
treating several types of cancer, its use has been 
limited due to toxicities. Several studies have found 
that during the early phase of CPT activation, there 
is an increase in reactive oxygen species (ROS) 
inside the cells, which causes subsequent elevation 
in the level of lipid peroxidation and decrease in 
reducing equivalents like GSH [5,6].

Vitamin E is a chain breaking antioxidant with proved 
non-enzymic antioxidant potential. There are a few 
reports on the concurrent use of vitamin E with 
chemotherapy to reduce the toxicity and increase the 
efficacy of the drug. Increased serum vitamin E 
levels have been reported to decrease lipid 
peroxidation and to protect fall in leukocyte count, 
hemoglobin level and mean osmotic fragility of 
erythrocyte [7]. This study is a preliminary evaluation 
of the hematological and biochemical profile of rats 
treated with CPT and to determine the extent to 
which vitamin E pretreatment could ameliorate the
detrimental effects induced by CPT.

MATERIALS AND METHODS

Drugs and chemicals: Camptothecin, α-tocopherol acetate, and reduced glutathione were 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 from Bharat Serum Pvt. Ltd, Thane, Navi Mumbai. The animals were maintained under the standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were fed with standard rat pellet 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 protocol: The rats were randomly divided into 4 groups consisting of six animals each. Group I served as control receiving saline throughout the experimental period. Group II rats were injected CPT (6 mg/kg body weight) dissolved in DMSO for four consecutive days, intravenously. Group III rats served as control group for vitamin E and received α-tocopherol (6 mg/kg 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. Blood was collected in EDTA for plasma and without anticoagulant for serum. Blood was processed further for RBC and WBC counts [8], differential count, platelet count [9], hemoglobin [10], glucose [11] and urea [12]. The serum levels of protein [13], uric acid [14], creatinine [15], bilirubin [15], cholesterol [16], triglycerides [17] and lipid peroxides [18] were also determined.

Statistical analysis: The Results were expressed as Mean ± Standard deviation (SD) for six animals in each group. Statistical significance of assay has been analyzed by unpaired Students t-test and given respective symbols in the tables.

RESULTS AND DISCUSSION

There were no distinctive clinical signs, mortality, or morbidity observed in any of the experimental groups during the experimental period. Control rats receiving vitamin E alone (Group III) did not show any significant change when compared with control rats (Group I), indicating that vitamin E does not have any adverse effect. Table 1 shows the results of the complete blood count. There is a significant decrease in number of RBCs (p<0.05) and WBCs (p<0.01) accompanied with a decrease in the levels of hemoglobin. Among the WBCs, there is significant decrease in the neutrophils. It is well known that myelo suppression and pancytopenia are characteristic features of chemotherapy. Actively proliferating progenitor cells in the bone marrow are sensitive to anti-cancer agents and are subjected to an irreversible removal process [19]. According to clinical studies of camptothecins, the principal dose-limiting toxicities are neutropenia, thrombocytopenia and anemia [4, 20-24]. In the present study, hematogenic effects of CPT including decreased RBC, WBC and platelets are in agreement with the results of above clinical studies. Premature death of RBCs as a result of oxidative injury can also contribute to the reduction in RBC count and a decrease in hemoglobin. Pretreatment with vitamin E showed beneficial effect by restoring the levels of RBCs, WBCs and hematocrit suggesting the ameliorative effect of vitamin E in preventing CPT induced bone marrow suppression. Our results are in agreement with those of Bipin Kumar [25] who had reported the modulatory role of vitamin E in radiation induced hematotoxicities.

A significant decrease in serum cholesterol and triglycerides levels was revealed in CPT treated rats
**Fig 1:** Effect of Camptothecin and Vitamin E on levels of triglyceride. Values are expressed as Mean ± SD for six rats in a group. Comparisons are made between: ‘a’ Groups I–II; ‘b’ Groups I and IV; ‘c’ Group II and IV. In fig, symbols represent statistical significance: ***P< 0.001.

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<th>Control</th>
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**Fig 2:** Effect of Camptothecin and Vitamin E on levels of cholesterol. Values are expressed as Mean ± SD for six rats in a group. Comparisons are made between: ‘a’ Groups I–II; ‘b’ Groups I and IV; ‘c’ Group II and IV. In fig, symbols represent statistical significance: ***P< 0.001, *P< 0.05.

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<th>Control</th>
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<td>Cholesterol (mg/dl)</td>
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**Fig 3:** Effect of Camptothecin and Vitamin E on levels of Serum lipid peroxides. Values are expressed as Mean ± SD for six rats in a group. Comparisons are made between: ‘a’ Groups I–II; ‘b’ Groups I and IV; ‘c’ Group II and IV. In fig, symbols represent statistical significance: *P< 0.05, **P< 0.01, NS- non significant.

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<th>Control</th>
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<td>Serum lipid peroxides (nmol/ml)</td>
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Table 1: Effect of Camptothecin and Vitamin E on complete blood count. Values are expressed as Mean ± SD for six rats in a group. 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, NS- Non significant, *** P<0.001

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<tr>
<th>Hematological Parameters</th>
<th>Group I (Control)</th>
<th>Group II (CPT)</th>
<th>Group III (Vitamin E)</th>
<th>Group IV (Vitamin E+CPT)</th>
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<td>Hemoglobin (mg/dl)</td>
<td>12.87 ± 0.63</td>
<td>11.750 ± 0.72a*</td>
<td>13.01 ± 0.78</td>
<td>12.417 ±0.61b NS, c NS</td>
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<td>RBC (million/mm³)</td>
<td>7.43 ± 0.46</td>
<td>6.77 ± 0.41a*</td>
<td>7.49 ±0.38</td>
<td>7.23 ± 0.33b NS, c NS</td>
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<td>WBC (per mm³)</td>
<td>12.466 ±1003</td>
<td>10.100±1252a**</td>
<td>12.521 ±1232</td>
<td>12.167 ±1674b NS, c *</td>
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<td>Neutrophile (%)</td>
<td>23.67 ± 3.83</td>
<td>14.67 ± 2.94a***</td>
<td>21.52 ± 2.20</td>
<td>21.67 ± 3.20b NS, c **</td>
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<td>Thrombocytes lakh/mm³)</td>
<td>9.27 ± 0.85</td>
<td>7.63 ± 0.61a**</td>
<td>9.10 ± 0.72</td>
<td>9.45 ± 0.86b NS, c **</td>
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<td>Lymphocyte (%)</td>
<td>78 ± 4.6</td>
<td>74.3 ± 3.9 a NS</td>
<td>77.5 ± 5.1</td>
<td>74.67 ±3.27b NS, c NS</td>
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In conclusion, the present investigation establishes that a good vitamin E profile is consistent with amelioration of toxicities induced by CPT. Often argument concerned with supplementation of antioxidants in chemotherapy arises. The basis of this disagreement is that many of the chemotherapeutic drugs induce the formation of oxygen-derived free radicals, the effect of which would be blocked by antioxidants. The anticancer effect of camptothecin however, does not depend on the formation of free radicals. Therefore, administration of antioxidants might reduce the side effects of camptothecin without compromising its efficacy. Further studies assessing the potential usefulness of α-tocopherol treatment in camptothecin induced toxicities on other organs and organ systems are warranted which may provide an effective way to improve their therapeutic efficacy.

REFERENCES

[5] Sen, N., Das, B.B., Ganguly, A., Mukherjee, T.,