International Journal of Scientific Reports
Roy R et al. Int J Sci Rep. 2015 May;1(1):45-51
http://www.sci-rep.com
pISSN 2454-2156 | eISSN 2454-2164

DOI: http://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20150200

Research Article

Toxicity profile of anticancer drugs in acute lymphoblastic leukemia and protective role of vitamin E on these toxicities in albino rats

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Received: 17 April 2015
Accepted: 25 April 2015

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ABSTRACT

Background: Objectives: (1) To study the spectrum of toxicity due to chemotherapy as per MCP841 protocol during the acute phase of treatment of patients with Acute Lymphoblastic Leukemia (ALL). (2) To study the same toxicities in albino rats and the protective role of vitamin E on these toxicities.
Methods: This was a prospective clinical study for one year, in the department of medical oncology and paediatric oncology which was augmented by experimental animal study for 5 months using albino rats. For clinical study, patients diagnosed as ALL taking treatment as per MCP841 protocol were taken. Patients were followed up every day to detect the development of any type of adverse reactions or toxicity symptoms. Laboratory tests done during the course of chemotherapy were reviewed for any abnormality. Animal study involved 18 albino rats; rats were divided into 3 Groups; Group 1 - control (n=6), Group 2 - antileukaemic treated rats (n=6), Group 3 - antileukaemic drugs and vitamin-E treated rats (n=6).
Results: In the clinical study major toxicities observed were haematological, metabolic, gastrointestinal, general infection, neurotoxicity, pancreatitis, pneumonitis and jaundice. Neurosensory toxicity presented as numbness in the extremities and hyperalgesia and myalgia. Histopathological examination of the internal organs of albino rats studied showed protection of vitamin E for the gastric toxicity, pancreatitis, cardiac toxicity, neuro toxicity and hepatic toxicity whereas there was no reduction in splenic and renal toxicities. In the case of haematological toxicity, protection was only minimal.
Conclusions: The animal study revealed the protective role of vitamin E on cytostatic drug induced toxicities.

Keywords: Acute lymphoblastic leukaemia (ALL), Antileukaemic drugs, Vitamin E (Vit. E), MCP841 protocol

INTRODUCTION

ALL is the most common malignancy in children. MCP841 is one of the ALL treatment protocols. The non-selective mechanism of action and resulting low therapeutic indices means that a high incidence of potentially severe toxicities must be tolerated to administer effective doses of anti-cancer drugs.[1,2]

These theoretical aspects, supported by epidemiological observations and lack of an experimental study of toxicities in animals using the MCP841 regimen prompted us to perform a scientific search of the toxicities and conduct a study with Vit.E as the antioxidant and the antileukemic drugs as the free radical inducer in albino rats.

METHODS

This was a prospective clinical study involving sixty-six ALL patients on treatment as per MCP841 protocol in the department of medical oncology and paediatric oncology. Distribution of patients in different phases of treatment is given in Figure 1.

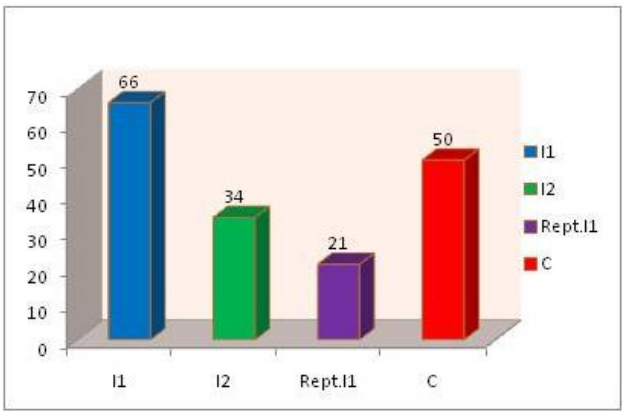

Figure 1: Distribution of patients in different phases of treatment.

Comparative study of spectrum of toxicities, graded according to WHO standards observed in ALL patients in the 4 phases of treatment as per MCP 841 regimen.

I_1 = Induction

I_2 = CNS directed therapy

Rep.I_1 = Repeat induction

C = Consolidation

The study was augmented by experimental animal models using albino rats, the drugs being supplied by medical oncology and paediatric oncology department.

In the clinical study patients diagnosed as ALL and receiving treatment for ALL during the Induction, CNS directed therapy. Intensification and consolidation phases of chemotherapy with vincristine, anthracyclines, cyclophosphamide, L-asparaginase, methotrexate, cytosine arabinoside and 6-mercaptopurine were included. Doses of the drugs based on the MCP841 protocol.

Although the specific approaches to patients in various risk groups may be somewhat different, modern ALL treatment regimens divide therapy into four main elements; viz. Remission induction, CNS preventive therapy, Consolidation and Maintenance therapy.[3]

Remission induction involves weekly doses of vincristine, anthracyclines, L-asparaginase and daily Prednisolone for 4-6 weeks.

CNS preventive therapy includes weekly intrathecal methotrexate and cranial irradiation. The remission achieved is consolidated with vincristine, cytosine arabinoside, cyclophosphamide and 6-mercaptopurine. This is followed by maintenance therapy with pulse doses of vincristine, anthracyclines, L-asparaginase and continuous oral methotrexate and 6-Mercaptopurine for a period of 18 months to 24 months.

Doses of drugs used

Daunorubicin IV 30 mg/m^2

Vincristine IV 1.4 mg/m^2

Prednisolone PO 40 mg/m^2

Asparaginase IM 6000 units/m^2

6-Mercaptopurine PO 75 mg/m^2

Cyclophosphamide IV 750 mg/m^2

Intrathecal methotrexate 12 mg/m^2

Cytosine arabinoside sc 100 mg/m^2

Reports of the base line investigations like haemoglobin, (Hb) total count (TC), Platelet count, Liver function Test (LFT), Renal Function Test (RFT), serum electrolytes, chest X-ray, ECG prior to therapy were collected. Patients were followed up every day to detect the development of any type of adverse reactions or toxicity symptoms at any time during the course of chemotherapy and findings were entered in the proforma sheet. Laboratory tests done during the course of chemotherapy were reviewed for any abnormality and relevant investigations pertaining to each system were done whenever indicated. After recording toxicities, they were graded according to WHO guidelines and all data collected were entered in the proforma sheet.

For experimental animal study 18 albino rats were divided into 3 Groups; Group 1 - control (n=6), Group 2 - antileukaemic drugs treated rats (n=6), Group 3 - antileukaemic drugs and vitamin-E treated rats (n=6). Duration of the study was 5 months.

Antileukaemic drugs which included vincristine (VCR), L-asparaginase (L-Asp), doxorubicin (ADR), prednisolone (PDN), were administered to Group 2 and Group 3 rats according to acute lymphoblastic leukemia treatment regimen (MCP841). All Drugs were given intraperitoneal (i.p.) except PDN which was given orally. Group-3 rats were given in addition to the antileukaemic drugs, Vit.E (100 mg/kg bodyweight/orally) daily.

Doses of drugs in animals

VCR -1.4 mg/m^2 IP

L-Asp - 6000 IU/m^2 IP

ADR - 30 mg/m^2 IP

PDN - 40mg/m^2 PO

Vit.E - 100 mg/kg body weight PO

Haematological investigations like Hb, TC, Platelet count, neurological examinations like testing for neuropathy were done during and at the end of the study.

Animals were sacrificed at the end and histopathological examinations of the internal organs were also carried out.

Statistical method used: Non-parametric technique - Kruskal Wallis - one way analysis of variance. The differences were considered to be significant when the calculated P <0.05.

Approval from institutional ethical committee was also taken.

RESULTS

Clinical study

Total No. of patients studied: 66

Total No. of male patients : 45 (66.18%)

Total No. of female patients: 21 (31.82%)

Age of patients studied: Ranging from 1 to 55 years

The predominant haematological toxicity in the intensive phase of treatment: grade 3 anaemia (42%) - Figure 2; grade 3 leukopenia (30.67%) - Figure 3.

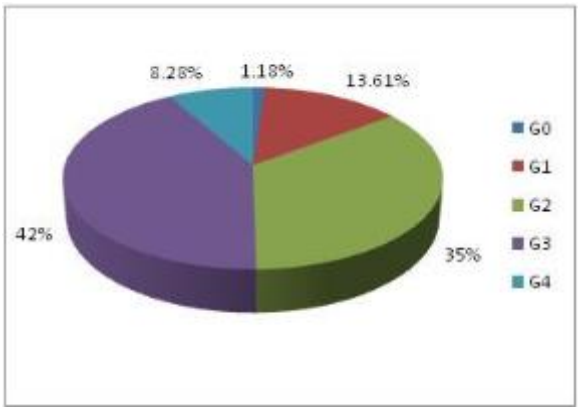

Figure 2: Distribution of different grades of anaemia during intensive phase of treatment.

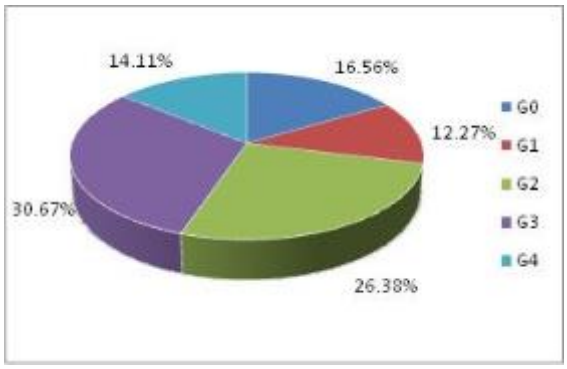

Figure 3: Distribution of different grades of leukopenia during intensive phase of treatment.

Grade 3 pancreatitis was observed in 6.06% of patients in the intensive phase of treatment (Table 1).

Grade 3 jaundice in 10.61% and grade 4 jaundice in 3.03% of patients was observed in the intensive phase of treatment (Table 2).

Table 1: Percentage distribution of patients with pancreatitis and the grades thereof.

Phase	Number of patients entered	Number of evaluable patients (%)	Grade					Total number of patients with toxicity
			Gr.0 (%) None	Gr.1 (%) Mild	Gr.2 (%) Moderate	Gr.3 (%) Severe	Gr.4 (%) Life threatening	(Gr.1-Gr.4) (%)
I_1	66	66 (100%)	58 (87.88%)	0	3 (4.55%)	4 (6.06%)	1 (1.52%)	8 (12.12%)
I_2	34	34 (100%)	32 (94.12%)	1 (2.94%)	1 (2.94%)	0	0	2 (5.88%)
Rpt.I_1	21	21 (100%)	19 (90.48%)	1 (4.76%)	0	1 (4.76%)	0	2 (9.52%)
C	50	50 (100%)	48 (96%)	1 (2%)	0	1 (2%)	0	2 (4%)
Total	171	171 (100%)	157 (91.81%)	3 (1.75%)	4 (2.34%)	6 (3.51%)	1 (0.58%)	

Grade 3 (severe) pancreatitis was observed in 6.06% of patients in the intensive phase of treatment

Table 2: Percentage distribution of patients with jaundice and the grades thereof.

Phase	Number of patients entered	Number of evaluable patients (%)	Grade					Total number of patients with toxicity
			Gr.0 (%) WNL	Gr.1 (%) -	Gr.2 (%) <1.5 x N	Gr.3 (%) 1.5 – 3xN	Gr.4 (%) >3 x N	(Gr.1-Gr.4) (%)
I_1	66	66 (100%)	52 (78.79%)	2 (3.03%)	3 (4.55%)	7 (10.61%)	2 (3.03%)	14 (21.21%)
I_2	34	34 (100%)	32 (94.12%)	0	2 (5.88%)	0	0	2 (5.88%)
Rpt.I_1	21	21 (100%)	19 (90.48%)	0	2 (9.52%)	0	0	2 (9.52%)
C	50	50 (100%)	47 (94%)	1 (2%)	1 (2%)	0	1 (2%)	3 (6%)
Total	171	171 (100%)	150 (87.72%)	3 (1.75%)	8 (4.68%)	7 (4.09%)	3 (1.75%)	

Grade 3 jaundice in 10.61% and grade 4 jaundice in 3.03% of patients was observed in the intensive phase of treatment

Minor toxicities observed in different phases

Neurocortical toxicity

I_1 = 6 (9.09%) patients

I_2 = 1 (2.94%) patients

Rept. I_1 = Nil

C = Nil

Neuromotor toxicity

I_1 = 12 (18.18%) patients

I_2 = 1 (2.94%) patients

Rept. I_1 = 3 (14.29%) patients

C = Nil

In the present study major toxicities observed were Haematological, Metabolic, Gastrointestinal, General Infection, Neurotoxicity, Pancreatitis, Pneumonitis and Jaundice.

Incidences of all the above toxicities were seen to be highest during I_1 phase.

Experimental animal study

Haemogram

Hb Values in 3 groups - 3rd week - day 19 was observed.

Difference between the mean values of Hb of group II &III = 0.483,

Technique used - ANOVA - one way analysis of variance

P = 0.898862; Not significant

Value of total count in 3 groups - 3rd week - day 19 was analysed (Table 3).

Table 3: Total count in animals.

Group	Mean total count	Variance	SD	F/H
I	11700	1.726×10^7	4154.56	H = 7.599
II	5316.667	5.850×10^6	2418.608	
III	8300	2.206×10^7	4696.275	

Difference between the mean values of total count of group II & III = 2983.333,

Non-parametric technique - Kruskal Wallis - one way analysis of variance

P = 0.022834; significant

Platelet count in 3 groups - 3rd week - day 19 was examined (Table 4).

Table 4: Platelet count in animals.

Group	Mean platelet count	Variance	SD	F/H
I	958333	1.484×10^{11}	385248.837	H = 6.593
II	451500	5.520×10^{10}	234941.482	
III	500000	8.663×10^9	93072.552	

Difference between the mean values of platelet count of group II & III = 48500

Non-parametric technique - Kruskal Wallis - one way analysis of variance

P = 0.037006; Significant

In the case of total count there is significant (P = 0.0228) difference between the groups (Table 3), pointing to a protection by Vit.E for group III. Similarly platelet count is much decreased in group II compared to group I, but the decrease is not much in group III showing significant protection by Vit.E (P = 0.037) (Table 4). Though the platelet count of control has not reached the normal range, there is significant difference between group II and group III.

Histopathological examination of the internal organs of albino rats studied

In our study out of the 6 animals in group II, 1 (16.67%) showed full thickness surface epithelial necrosis in stomach (Plate 1 & 2) The necrosis could be due to the drug induced changes in the gastric mucosa, and difference in severity in necrosis could be due to Vit.E protection in group III (Plate 2).

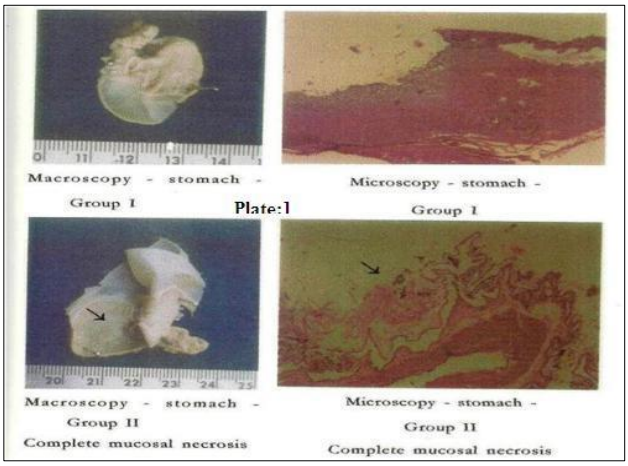

Figure 4: Plates: 1&2 - Histopathology of stomach.

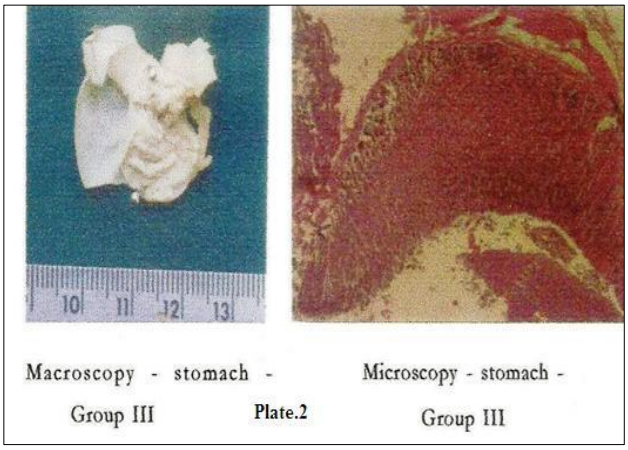

Figure 5: Plates: 2 - Histopathology of stomach.

There is protection for pancreas as evidenced by pancreatitis in 1 out of 6 animals (16.67%) in group II whereas none in group III (Plate 3 & 4). L-Asp is reported to produce pancreatitis.

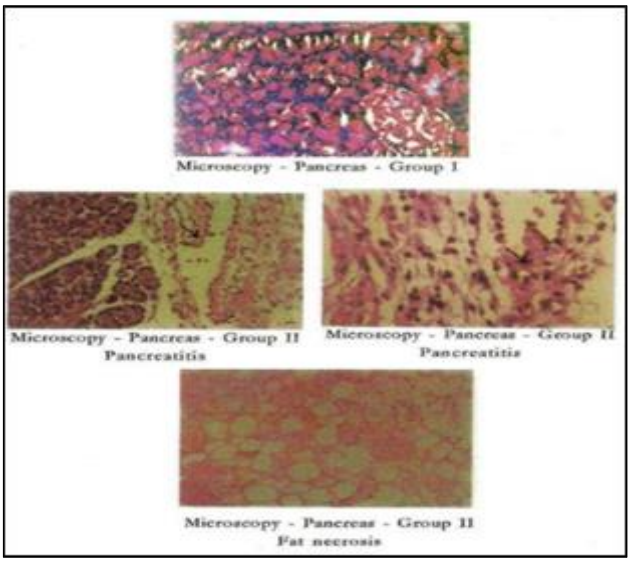

Figure 6: Plates: 3 - Histopathology of pancreas.

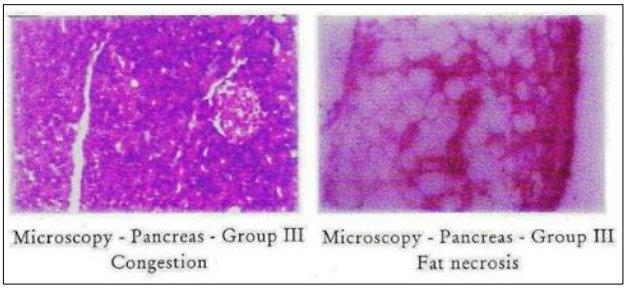

Figure 7: Plate: 4 - Histopathology of pancreas.

Pericarditis was seen in 2 out of 6 animals (33.33%) in group II and none observed in group III (Plate 5). Doxorubicin causing acute pericarditis in early stages of its administration is a reported fact. Thus from our study we concluded there was protection for heart also by Vit.E.

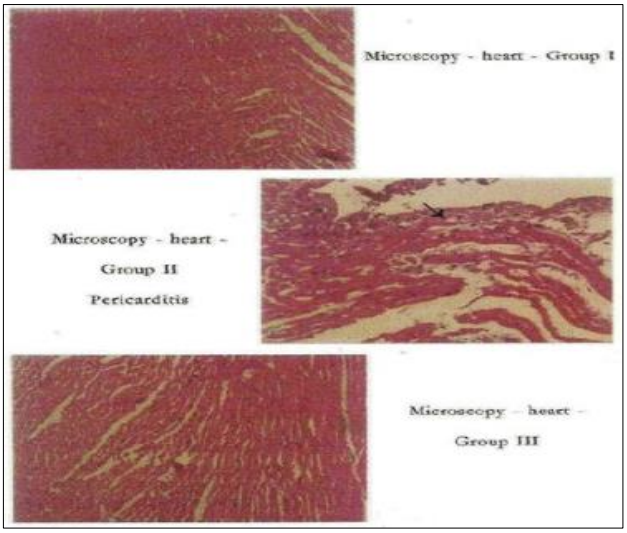

Figure 8: Plate: 5 - Histopathology of heart.

Histopathological examination of the liver showed marked toxicities, especially in group II such as congestion, Zone III necrosis, haemorrhage, cystic changes. Group III rats only showed mild congestion (3/6) and fatty change (1/6) (Plate 6) evidencing the scavenging role of Vit.E on liver toxicity.

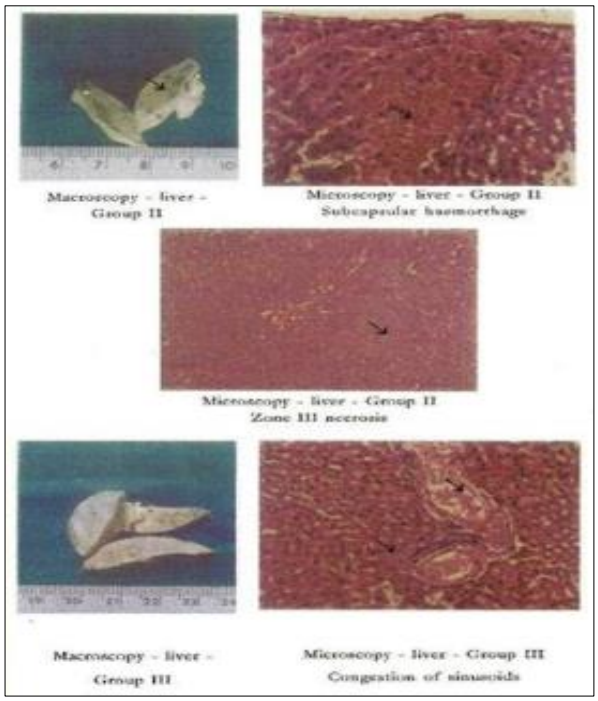

Figure 9: Plates: 6 - Histopathology of liver.

DISCUSSION

Anticancer treatment is generally associated with toxicity to healthy tissues. One of the reasons for this unpleasant association is that anticancer agents have been mostly selected on the basis of an empirically established toxicity towards cancer cell lines and rapidly growing tumours in animal models and not on the basis of a sophisticated intervention in tumour-specific biology.[4]

Hepatic fibrosis and even cirrhosis are reported to occur in about 20% of patients who receive chronic low dose of MTX.[5]

The primary toxic effects of MTX are myelosuppression and oro intestinal mucositis, which occur 5 to 14 days after the dose.

The clinical toxicity of VCR is mostly neurological [6].The delayed functional cardiotoxic effects of repeated treatment with Doxorubicin, 1.5 mgm/kg IV once a week for 5 consecutive weeks in rats were investigated by Cirillo et al.[7] Histopathologic investigations indicated alterations in ventricular myocytes. In addition atrial lesions were evident in Doxorubicin-treated rats. Wahab MH et al.[8] studied the modulatory effects of melatonin and Vit.E. 250 mg/kg/day x 15 days, PO starting 24 hour prior to Doxorubicin treatment significantly increased cardiac contents of total protein, glutathione (GSH), superoxide dismutase (SOD) by 23%, 26% and 39% respectively, while the cardiac content of malondialdehyde (MDA) was decreased by 35% compared with doxorubicin only treated group.

Two categories of toxic effects result from the therapeutic use of corticosteroids:[9] those resulting from withdrawal of steroid therapy and those resulting from continued use of supraphysiological doses.

Smeland et al.[10] found acute renal and hepatic toxicity as evidenced by severe morphological findings, during their investigations to elucidate the mechanism for Methotrexate induced renal and hepatic toxicity.

Recent evidences suggest that administration of cytostatic agents lead to generation of free radicals *in vitro* and *in vivo*.[11] *In vitro* and experimental animal studies have shown that the co-administration of free radical scavengers did not reduce anti-tumour effect of cytostatic agents and that the survival of animals was increased compared to the survival of animals that received chemotherapy alone.[12]

Protective role of Vit.E on liver and stomach in rats was studied by Canturk NZ et al.[13] The authors found that macroscopic and microscopic mucosal injury were significantly greater in the control than the Vit.E pre-treatment group ($P < 0.05$).

Antioxidants are believed to quench free radicals.[14]

MCP - 841 Protocol, the Multi Center Protocol used in this study is an aggressively antileukemic treatment regimen in India and has been the subject of intensive study earlier. The higher incidence rate of all the toxicities in the induction phase could be attributed to the aggressive nature of the regimen. However, literature reports that protocols using this 4 drug induction combination with intensive consolidation and maintenance therapy, uniformly demonstrate improved overall remission duration, even for high risk patients.

Our experimental animal study showed the toxicity in albino rats and the protective role of Vit.E on drug induced toxicities.

CONCLUSION

The results of the experimental animal study revealed the protective role of Vit.E, the most important antioxidant, on cytostatic drug induced toxicities. Among the toxicities Vit.E could give protection for the gastric toxicity, pancreatitis, cardiac toxicity, neuro toxicity and hepatic toxicity in albino rats, whereas there was no reduction in splenic and renal toxicities. In the case of haematological toxicity, protection was only minimal. Even then our study points to the role of Vit.E as a free

radical scavenger in the chemotherapy induced toxicities. It therefore warrants further extensive experimental animal study and clinical evaluation. Approaches to reduce or prevent chemotherapy induced toxicity include: alternating the duration and route of administration, the use of drug carriers, analogues and prodrugs,[15] the use of growth factors to enhance the recovery of the remaining haematopoeitic progenitor/stem cell population and treatment with rescue agents or cytoprotectants.

Essence & achievements of these studies are as follows

The spectrum of toxicities, the phases of treatment in which they occur and the severity thereof, according to the gradings stipulated by WHO, have been closely scrutinized and comparative analysis of predominant toxicities in the 4 phases has been performed and catalogued.

From the view point of toxicities, the status of MCP 841 has been satisfactorily demonstrated in our study to be the preferential regimen for antileukemic therapy.

The induction of toxicities in animal models and the beneficial effect of prophylactic use of Vit.E as an anti-oxidant was investigated and confirmed. Implementation of Vit.E administration along with antileukemic regimen would however be possible only after further extensive randomized clinical trials.

The goal for the future should be to design therapy which is effective and has high specificity for the biology of cancer, efficiently targeting leukemic cells and thus sparing the normal cells from the toxicities.

ACKNOWLEDGMENTS

Authors are immensely obliged to Mr. S. Muraleedharan Nair, MSc, Medical Statistics, CERTC, Medical College, Trivandrum for lending his specialized help in the statistical analysis.

Key message

Role of vitamin E as a free radical scavenger in the chemotherapy induced toxicities.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Roy R, Hema CG, Geetha N. Toxicity profile of anticancer drugs in acute lymphoblastic leukemia and protective role of vitamin E on these toxicities in albino rats. Int J Sci Rep 2015;1(1):45-51.