COMBINED EFFECT OF CYCLOPHOSPHAMIDE AND EXTRACTS OF *CROTALARIA* AND *SENECIO* PLANTS ON EXPERIMENTAL TUMOURS

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Summary : The combined effect of cyclophosphamide (CTX) and extracts of six piants belonging to *Crotalaria* and *Senecio* genera was assessed on experimental transplantable S180 (both ascitic and solid forms) tumour. Successive petroleum ether and methanolic extracts from these plants were obtained. The combined administration of CTX and petroleum ether extract of *C. albida* and the methanolic extracts of *C. albida*, *S. chrysanthemoides*, *S. densiflorus* and *S. jacquemontianus* led to prolonging the life span of S180 (ascitic) tumour bearing mice. The data indicate that the most effective extract in combination with CTX was the methanolic extract of *S. chrysanthemoides*. The extracts alone had no effect on survival of tumour-bearing mice. The same extracts and the same combinations had no effect on S180 solid tumour.

Key words : crotalaria, senecio, plant extracts, cyclophosphamide, potentiation, S180 tumour, antitumour activity, combination therapy

INTRODUCTION

Combinations of anti-cancer drugs belonging to different classes where the toxicity does not overlap are extensively used in clinics. In recent times, anti-cancer chemotherapy combined with non-anti-cancer drugs is looked upon as one of the approaches towards enhancement of the efficacy of chemotherapeutic agents in the treatment of malignant diseases (8). For example, polyene antibiotics and probencid were used in an attempt to increase cell membrane permeability for anti-cancer chemotherapeutic agents (1,6). Potentiation of anti-cancer agents by amphotericin B has been reported (7). Both saponin and digitonin could make plasma membrane permeable to various substances without impairing the function of the intracellular organelles (4). Cimetidine

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a known histamine H_2 blocker increases cyclophosphamide (CTX) activity (2) Tsuruo *et al.* (9) demonstrated that calcium channel blockers enhance *Vinca* alkaloids and Adriamycin cytotoxicity *in vitro* and *in vivo* in murine tumours as well as *in vitro* cytotoxicity in human leukaemic cell lines.

In the present communication we have examined the combined effect of extracts from *Crotalaria* and *Senecio* plants and CTX on Sarcoma 180 (both ascitic and solid form).

MATERIAL AND METHODS

The shade dried plant powder of the six plants undertaken for study was extensively extracted with petroleum ether (Ext. 1) and methanol (Ext. 2) in succession in a Soxhlet apparatus. The solvents were removed under reduced pressure and the corresponding extracts were obtainad (Table I). The extracts were suspended in normal saline containing 2% Tween 80.

Cyclophosphamide (Biochem Pharmaceutical Industries, Bombay) was solubilized in distilled water.

Tumour transplantation and measurements: The ascites cell form of Sarcoma 180 was grown in Swiss mice and maintained by serial passages ip. Transplantation was carried out with donor animals bearing 7-day-old tumour growth. *In vivo* activity was performed as described in the standard protocol (3). Mice were weighed during the course of the experiment, i.e. on days 1,5 Average body weigh difference was used as an indication of host toxicity Dosage levels of each extract administered over a range of 100-400 *mg/kg* on days 1,5,9 beginning 24 hr after tumour implantation. CTX was administered simultaneously. Determination of the sensitivity of ascitic neoplasm to the treatment was based upon prolongation of survival time. Survival time of tumour bearing mice was scored and the ratio of median survival time (MST) of treated to control mice (T/C) as percentage was calculated. T/C% value of $\geqslant$ 125 was taken to demonstrate activity.

Inhibition of the growth of S180 (solid) tumour by test materials was examined as described in the previous paper (5). Briefly groups of 6 mice weighing 22-26 g were injected sc with the ascitic cells in the axillary region. Combination chemotherapy was started next day of tumour inoculation. Treatment (ip) was given on days 1,5,9. CTX was administered simultaneously. Body weights were recorded on days 1 and 5. At the end of the treatment the animals were killed. Tumours were dissected out. Tumour

weights were noted. Percent tumour weight inhibition (TWI%) was calculated as $(1-T/C)\times 100$ where T is the average tumour weight of treated group and C is the average tumour weight of control group. TWI% $\geq$ 58 was taken to indicate anti-tumour activity.

RESULTS AND DISCUSSION

The work deals with six plants belonging to *Crotalaria* and *Senecio* genera as shown in Table I. Both the successive petroleum ether and methanolic extracts of these plants were studied in combinations with CTX. Survival time of animals are given in Table II. It depicts that the petroleum ether and methanolic extracts of *C. albida* enhance CTX activity when administered simultaneously. The results also showed that the methanolic extracts of *S. chrysanthemoides, S. densiflorus* and *S. jacquemontianus* were similarly effective (Table III). It thus appeared that even in the effective combined treatment maximum potentiation of CTX activity was obtained with methanolic extract of *S. chrysanthemoides*. However, there was no difference in survival between the control groups and extracts treated groups, all animals died within 15 days after tumour cell injention (data not incorporated).

In a study carried out on S180 (solid) tumour, administration of the extracts from the six plants had no effect on the growth of the tumour. The combined administration too, failed to produce tumour inhibition (data not shown).

This communication shows a beneficial effect of combination of certain plant extractives with cyclophosphamide Mechanism of this effect is unclear at this stage of work. It would be of interest to attempt further fractionation of active extracts.

TABLE I : Plant products studied for synergistic effect with cyclophosphamide.

	Plant	*Part used for extraction*	*% Yield*	
			Petroleum ether	*Methanol*
CA	*Crotalaria albida*	Leaves	5.8	17.0
CJ	*Crotalaria juncea*	Whole plant	3.1	15.2
SC	*Senecio chrysanthemoides*	Plant	1.9	16.4
SD	*Senecio densiflorus*	Plant	1.6	13.4
SJ	*Senecio jacquemontianus*	Roots	2.2	8.3
SR	*Senecio rufinervis*	Plant	2.6	9.8

TABLE II : Response of S180 (ascitic) tumour to the combination of CTX and extracts of *Crotalaria* plants.

*Material**	*Dose (mg/kg)*	*MST** (days)*	*T/C%*
CTX	55	15/11.5	130
CTX+CA1	55+400	19.5/11.5	169
CTX	55	15/11.5	130
CTX+CA2	55+400	19/12	158
CTX	55	19/12	158
CTX+CJ1	55+200	13.5/13	104
CTX	55	16/11.5	139
CTX+CJ2	55+400	13/12	108

*1. Petroleum ether extract 2. Methanolic extract
**MST of treated animals (T)/MST of control animals (C)

TABLE III : Response of S180 (ascitic) tumour to the combination of CTX and extracts of *Senecio* plants.

*Material**	*Dose (mg/kg)*	*MST** (days)*	*T/C%*
CTX	55	16/11.5	139
CTX+SC1	55+400	16.5/12	138
CTX	55	16/11.5	139
CTX+SC2	55+400	26/12 (P<0.001)	217
CTX	55	16/12	133
CTX+SD1	55+400	12.5/11.5	109
CTX	55	16/12	133
CTX+SD2	55+400	20.5/11.5 (P<0.05)	178
CTX	55	19/13.5	140
CTX+SJ1	55+100	19/14	136
CTX	55	15/11.5	130
CTX+SJ2	55+400	22.5/12 (P<0.01)	188
CTX	55	17/11.5	148
CTX+SR1	55+400	13.5/11.5	117
CTX	55	17/11.5	148
CTX+SR2	55+400	17/11.5	148

*1. Petroleum ether extract 2. Methanolic extract
**MST of treated animals (T)/MST of control animals (C)

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