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Antitumour Activity of Plumbagin against Dalton's Ascitic Lymphoma

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The antitumour activity of plumbagin (2-methyl 5-hydroxy, 1,4 napthoquinone) has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice. A significant enhancement of mean survival time of Plumbagin treated tumour bearing mice was found with respect to control group. Plumbagin treatment was found to enhance peritoneal cell counts. When these Plumbagin treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. At 14 days after transplantation, Plumbagin treated groups were able to reverse the changes in the haemotological parameters, protein and PCV consequent to tumour inoculation.

PLUMBAGIN[1,2] (2-methyl 5-hydroxy 1,4 napthoquinone) isolated from the roots of **Plumbago zeylancia**[3] (Plumbaginaceae) has been reported for its anticancer activity against fibrosarcoma induced by methyl cholanthrene and P 388 lymphocytic leukemia[4]. The LD_{50} of plumbagin was approximately 10 mg/kg (oral and i.p.) in mice[5]. The present study is focused on the evaluation of anticancer activity of plumbagin against Dalton's Ascitic Lymphoma in mice.

MATERIALS AND METHODS

Plumbagin, (Sigma chemical Co., USA) was dissolved in phosphate buffer (pH-7.4) and used in the present study.

Swiss albino mice (20-24 g) were used throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water *ad libitum*. Dalton's Ascitic Lymphoma (DAL) cells were obtained through the courtesy of Cancer Research Institute, Adayar, Madras. DAL cells were maintained by weekly intraperitoneal (i.p.) inoculation of 10^6 cells/mouse.

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Effect of plumbagin on survival Time

Animals were inoculated (i.p.) with 2-3 x 10^5 cells/mouse on day 0, and treatment with Plumbagin started 24 h after inoculation, at a dose of 2 mg/kg/day i.p. (Group-A). The control group (Group B) was treated with the same volume of 0.9% saline. All treatments were continued for nine days. Median survival times (MST) for each group, containing 9-11 mice, were noted. The animals surviving more than 60 days were considered to be cured. The antitumour efficacy of plumbagin (2 mg/kg/day i.p., for 9 days) was compared with that of 5 Fluorouracil (5 FU: 20 mg/kg/day i.p. for 9 days.) MST were noted with reference to control. Survival times of treated groups (T) were compared with those of control groups (C) using the following calculation.

$$\text{Increase of life} = T/C\ \% = \frac{\text{MST of treated group} \times 100}{\text{MST of control group}}$$

Effect of plumbagin on tumour cell growth

Studies on *in vivo* tumour cell growth inhibition with plumbagin carried out under similar experimen-

Table 1: Effect of inoculation with plumbagin or 5FU treated DAL

Treatment	MST (days)	Increase in Life Span T/C%
Plumbagin (2mg/kg i.p.)	37	160.8
5 FU (20mg/kg i.p.)	40	173.9
Saline	23	100.00

Table 2: Effect of plumbagin (2mg/kg/day i.p.) treatment on enhancement of Peritoneal cells in normal mice

Experiment	Number of Peritoneal cells/ mouse x 10^6
Control	$5.9\pm0.8\times10^6$
Treated once	$8.9\pm1.8\times10^6$
Treated twice on two consecutive days	$12.7\pm2.1\times10^6$

tal conditions as stated above, using the dose of 2 mg/kg/day for 6 days. Animals were sacrificed on day 7 after transplantation and tumour cells were collected by repeated intraperitoneal wash with 0.9% saline, viable tumour cell counts (Trypan blue test) were made using a haemocytometer. Total number of viable cells per animal of the treated group was compared with those of the control group.

Effect of pumbagin on normal peritoneal cells

Three groups of normal mice (n = 5 each) were used for the study. One group was treated once with 2 mg/kg i.p of plumbagin, while the second group received the same treatment for two consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24 h after treatment for each of the treated groups and compared with those of the untreated group.

Effect of plumbagin treatment prior to inoculation with DAL cells

Two groups of animals (n = 5 each) were used for the study, one group was treated with 2 mg/kg/ip/day of pumbgain for a period of two days while the untreated group served as control. After 48 h both the treated and untreated groups were inoculated (i.p.) with 10^5 DAL cells/mouse. Animals were sacrificed three days after transplantation and tumour cells were counted in both the groups.

Haematological studies

In order to detect the influence of plumbagin on the haemotological status of DAL bearing mice, comparison was made amongst three groups (n=5) of mice on the 14th day after transplantation. The three groups comprised (1) tumour bearing mice (2) tumour bearing mice treated with plumbagin 2 mg/kg/day i.p. (9 days) and (3) normal mice. Blood was drawn from each mouse in the conventional way and the white blood cell count (WBC), red blood cell count, haemoglobin (Hb), protein and packed cell volume (PCV) were determined[6,7]. The average of 5 determination was computed.

RESULTS

The effect of plumbagin on the survival of tumour bearing mice showed the MST for the control group to be 23 days, while it was 37 days and 40 days for the groups treated with plumbagin (2 mg/kg/day i.p.) and 5FU (20 mg/kg/day i.p.) respectively.

On day 7 after transplantation, the i.p. DAL cell count (control) was $2.12 \pm 0.21 \times 10^8$ cell/mouse. This value was significant ($P<0.01$) when compared to that of control. The sizes of the DAL cells harvested from treated mice were appreciably smaller with respect to the control cells.

The average number of peritoneal exudate cells per normal mouse was found to be $5.9 \pm 0.8 \times 10^6$. Plumbagin (2 mg/kg i.p.) treatment increased the

Table 3 : Effect of Plumbagin (2mg/kg/day i.p.) on Haematological Parameters

	Hb (g%)	Total RBC cells/ml x10^{10}	Total WBC cells/ml x10^{6}	Protein g%	PCV (mm)	Differential count		
						Lympho cyte	Neutro phil	monocyte
Normal mice	16.3±0.6	1.40±0.5	6.7±0.5	8.15±0.3	17±0.6	68.0±2.0	30±2	2±1.0
Tumour bearing mice (14 days)	13.2±0.9	1.31±0.3	15.5±0.7	11.25±0.5	39±0.4	26.0±3.1	71±5	3±0.9
Treated tumour bearing mice	16.1±0.5	1.46±0.4	6.8±0.2	9.10±0.6	20±0.5	61.0±1.0	34±1	5±1.0

number of peritoneal cells as shown in Table-2. Single treatment enhanced peritoneal cells to 8.9 ± 1.8 x 10^{6}. While two consecutive treatments enhanced the number to 12.7 ± 2.1 x 10^{6}.

Haematological parameters of (Table-3) tumour bearing mice on day 14 were found to be significantly altered from the normal group. The total WBC count, protein and PCV were found to be increased with a reduction of the haemoglobin content of RBC. The total number of RBC showed a modest change. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, plumbagin (2 mg/kg/day i.p.) treatment could restore those altered parameters to normal.

DISCUSSION

The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the animal and disappearance of leukemic cells from blood.[8,9] The above results demonstrated the antitumour effect of plumbagin against DAL in swiss albino mice. A significant enhancement of MST was found. Our observations of inhibition of **in vivo** tumour cells growth after plumbagin treatment appeared to correlated with the finding of enhancement of survival time by plumbagin with respect to control. The harvested viable cells after plumbagin treatment showed morphological changes as revealed by a reduction in the size of cells. Plumbagin treatment was found to enhance peritoneal cell counts. (Table-2).

Analysis of the haemotological parameters showed a minimum toxic effect in the mice which were cured by plumbagin treatment. At 14 days after transplanation, plumbagin treated groups were able to reverse the changes in the haemotological parameters consequent to tumour inoculation.

The possible mechanism of antitumour action of plumbagin against DAL cells may be due to radiomimetic, nucleotoxic and cytotoxic effect. It closely correlates with the theory that plumbagin acts in a manner similar to that of a spindle poison and inhibits cell mitosis[10].

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