### CHAPTER IV

# MAINTENANCE OF TUMOR CELL LINE DALTON'S LYMPHOMA AND DETERMINATION OF LIFE SPAN OF WHITE MICE

## MAINTENANCE OF TUMOR CELL LINE DAL AND THE DETERMINATION OF LIFE SPAN OF C<sub>3</sub>H / HE MICE

#### Description of Ascites tumor Dalton's lymphoma:

The occurrence of free tumor cells in certain neoplastic exudates was detailed, and the term ascites tumor has generally been applied to the exudates accumulation in the peritoneal cavity containing a large proportion of free tumor cells. Such tumors with the state of a nearly pure culture, offer a great many advantages over the use of solid tumors. Generally the ascites tumors have been developed experimentally by implanting solid tumor preparations intraperitoneally into genetically related strains of animals. After a period of time, samples of the abdominal fluid are removed and reinoculated intraperitoneally into a fresh group of animals (Goldie, and Felix, 1951; Klein, 1951). By suitable modifications in procedure it is possible to shift the ratio between solid growth and free cells in the peritoneal fluid (Klein et al., 1951). A systematic method of producing ascitic from solid tumors has been described (Klein et al., 1951) and shown to be applicable to a wide variety of experimental neoplasms.

The existence of individual, free tumor cells makes possible the experimental demonstration of the transmissibility of a single tumor cell.

Dalton's lymphoma (DAL) is a transplantable T cell lymphoma of spontaneous origin (Klein, 1951 and Goldie, 1951). DAL growth has been shown to be associated with a concomitant inhibition of humoral and cell-mediated immune responses, involving the abrogation of the functions of macrophages, B and T cells (Parajuli and Singh, 1977; Kumar et al., 1994; Kumar et al., 1995 and Priare et al., 1995). Thus the use of DAL has been proved to be successful.

#### Maintenance of Dalton's lymphoma:

DAL cells were maintained by weekly intraperitoneal (i/p) inoculation of  $10^6$  cells / mouse (Gothoskar and Ranadive, 1971; Majumdar et al., 1997). Transplantable ascites DAL tumor was maintained in  $C_3$  H / He male mice (as described in earlier chapter II) of 9-10 weeks age, weighing 18-22 g by serial i. p. transplantation of  $1 \times 10^7$  viable tumor cells. After  $7^{th}-15^{th}$  day of DAL inoculation in white mice ascites fluids were fully formed. Now these fluids were transferred to new mice with the help of a syringe, which were diluted with phosphate – buffered saline, (PBS). 0.25 ml of DAL was injected at the lower part of the belly in mice. Tumor transplanted animals survived for 21-23 days with a mean survival time of 22 days.

#### Determination of life span:

Using BRH<sub>2</sub> on Dalton's lymphoma implanted in mice it has been observed that the 100 mg / kg / bw on 1<sup>st</sup>, 5<sup>th</sup> and 9<sup>th</sup> day (i/p) can increase the life

span above 70% (23 days to 40 days) (*Table.4.1*). The dose 100 mg / kg / bw are used for our experiment.

Table: 4.1: Survival of C<sub>3</sub>H / He mice bearing Ascites Dalton's lymphoma cells, treated with BRH<sub>2</sub> [in carboxymethyl cellulose (CMC) used as vehicle]

Group	Dose	Experiment-1		Experiment- 2		Experiment-3	
	mg/kg/bw	MST	%	MST	%	MST	%
*		(Days)	ILS	(Days)	ILS	(Days)	ILS
Control		11	-	10.5	-	11.5	-
(DAL + CMC)							
BRH <sub>2</sub> (Treated)	50	15	36.3	15.5	47.6	16	39
	100	19.5	77.3	19	80.9	20	73.9
	150	16.0	45.5	16.5	57.1	17	47.8
	200	14.5	31.8	16	52	16.5	43
	400	4	Toxic	3.5	Toxic	3.5	Toxic

Drug administered i.p. on days 1, 5 and 9

% ILS = % Increase in life span

MST = Median survival time.