Effect of the Methanolic Extract of *Glinus lotoides* on Dalton's Ascitic Lymphoma

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The antitumour activity of the methanolic extract of *Glinus lotoides* (MGL) has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice. A significant enhancement of mean survival time of tumour bearing mice and peritoneal cell count in normal mice was observed with respect to the control group. When these MGL treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. After 14 d of inoculation, MGL is able to reverse the changes in the haematological parameters, protein and packed cellular volume consequent to tumour inoculation.

Key words *Glinus lotoides*; Dalton's ascitic lymphoma; haematological study; life span

Many naturally occurring substances were tested for anticancer activity on experimental animals resulting in the present availability of some 30 effective anticancer drugs.¹⁾ The present study is focused on evaluation of the antitumour activity of the methanolic extract of *Glinus lotoides*²⁾ (traditionally used for various abdominal disorders) against Dalton's ascitic lymphoma.

MATERIALS AND METHODS

Materials Whole parts of *Glinus lotoides* (Aizoaceae) collected in and around the Tiruchirappalli District of Tamil Nadu, India were used for the extraction. They were dried in the shade, pulverised and packed into a soxhlet apparatus (500 g) and subjected to hot continuous percolation using methanol (1.51) for 36 h.³⁾ The extract (5.2%, w/v) was dried then suspended in 5% gum acacia for the phytochemical and pharmacological studies.

Animals Swiss albino mice (20—26 g) were used throughout the study. They were housed in standard microton boxes and were given standard laboratory diet and water *ad libitum*.

Cells Dalton's ascitic lymphoma (DAL) cells were obtained courtesy of the Cancer Research Centre (CRC), Adyar, Chennai (originally brought from Prof. G. Klein, Stockholm, Sweden) and given by intraperitoneal transplantation of 10⁶ cells/mouse.^{4,5)}

Effect of MGL on Survival Time⁶⁾ Animals were inoculated with 10⁶ cells/mouse on day 0 and treatment with MGL started 24 h after inoculation, at a dose of 50 mg/kg/d, i.p. (group-A). The control group (group-B) was treated with the same volume of 0.9% sodium chloride solution. All treatments were carried out for 9 d. Mean survival times (MST) of each group, containing 10 mice, were noted. The antitumour efficacy of MGL was compared with that of 5-Fluorouracil (5-FU: 20 mg/kg). Survival times of treated group were compared with control group using the following equation.⁷⁾

$$\text{increase of life} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100$$

Tumour Cell Growth⁶⁾ Studies on *in vivo* tumour cell growth inhibition with MGL were carried out under similar

experimental conditions as stated above, using a dose of 50 mg/kg/d for 6 d. Animals were sacrificed on day 7 after transplantation and tumour cells were collected by repeated intraperitoneal wash with 0.9% NaCl. Viable tumour cell counts (Trypan blue test) were made with a haemocytometer.

Effect of MGL on Normal Peritoneal Cells⁶⁾ Three groups of normal mice ($n=5$) were used for the study. One group was treated with 50 mg/kg i.p. of MGL and the second group received the same treatment for 2 consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24 h after treatment for each treated group and compared with those of the untreated group.

Effect of MGL on Haematological Parameters⁶⁾ In order to detect the influence of MGL on the haematological status of DAL bearing mice, comparison was made amongst three groups ($n=5$) of mice on the 14th day after inoculation. The 3 groups comprised (1) tumour bearing mice (2) tumour bearing mice treated with MGL (50 mg/kg/d, i.p.) for 9 days and (3) normal mice. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count (using a Neubauer counting chamber), haemoglobin (using Shali's acid haematin method), protein, differential count and packed cellular volume (using Wintrobe's method) were determined.^{8—10)} All the results were analysed by analysis of variance.¹¹⁾

RESULTS

The preliminary phytochemical screening reveals the presence of flavanoids in the methanolic extract of *Glinus lotoides*. The effect of MGL on the survival of tumour bearing mice showed MST for the control group to be 23 d, while it was 30 and 40 d for the groups treated with MGL (50 mg/kg/d, i.p.) and 5 FU (20 mg/kg/d, i.p.) respectively. (Table 1).

The average number of peritoneal exudate cells per normal mouse was found to be $6.8 \pm 0.8 \times 10^6$. MGL (50 mg/kg) treatment increased the number of peritoneal cells as shown in Table 2. Single treatment enhanced the peritoneal cells to $8.1 \pm 0.9 \times 10^6$ while two consecutive treatments enhanced the number to $8.4 \pm 1.0 \times 10^6$.

Haematological parameters (Table 3) of tumour bearing mice on day 14 were found to be significantly altered from

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Table 1. Effect of MGL Treatment on the Survival of Tumour Bearing Mice

Treatment	MST (d)	Life span (%)
Saline (control)	23±1.02	100
5-FU (20 mg/kg, i.p.)	40±0.70	173.9
MGL (50 mg/kg, i.p.)	30±1.08*	130.4

* $p<0.001$ vs. control. Number of animals used=10 in each group. Days of drug treatment=9. Values were expressed as mean±S.E.

Table 2. Effect of MGL (50 mg/kg, i.p.) Treatment on Enhancement of Peritoneal Cell Count in Normal Mice

Experiment	Number of peritoneal cells (1×10^6)/mouse
Control	6.8±0.8
Treated once	8.1±0.9*
Treated twice on two consecutive days	8.4±1.0*

* $p<0.001$ vs. control. Number of animals used=5 in each group. Values were expressed as mean±S.E.

Table 3. Effect of MGL (50 mg/kg/d, i.p.) on Haematological Parameters in Mice

	Hb (g%)	RBC (million/mm ³)	WBC (cells/mm ³)	Protein (g%)	PCV (mm)	Differential count %		
						Lymphocytes	Neutrophils	Monocytes
Normal mice	15±0.47	4.1±0.22	8.068±38	8.8±0.4	16±0.66	68±1.3	30±2.1	2±0
Tumour bearing mice (14 d)	10±0.33	2.8±0.08	68.000±180	13.2±0.2	26±0.54	30±1.5	69±1.6	1±0
Treated tumour bearing mice	12.9±0.38	3.8±0.16*	15.550±108*	9.7±0.8*	20±0.83	70±1.6	28±1.3	2±0

* $p<0.001$ vs. control. Number of animals=5 in each group. Days of drug treatment=9.

the normal group. The total white blood cells count, protein and packed cell volume were found to be increased with a reduction of the haemoglobin content of red blood cells. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, MGL (50 mg/kg/d, i.p.) treatment could change these altered parameters to near normal.

DISCUSSION

The reliable criterion for judging the value of any anti-cancer drug is the prolongation of lifespan of the animal¹² and reduction of WBC from blood.¹³ The above results demonstrated the antitumour effect of MGL against DAL in Swiss albino mice. A significant enhancement of MST was found. The harvested viable cells (trypan blue method) after MGL treatment showed morphological changes as revealed by the reduction in size of the cells.

To evaluate whether MGL treatment indirectly inhibited the tumour cell growth, the effect of MGL was examined on the peritoneal exudate of normal mice. Normally each mouse contain about 5×10^6 intraperitoneal cells, 50% of which are macrophages. MGL treatment was found to enhance peritoneal cell counts. When these MGL treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. These results demonstrated the indirect effect of MGL on DAL cells, probably mediated through enhancement and activation of macrophages or through some cytokine product inside the peritoneal cavity produced by MGL treatment.

Analysis of the haematological parameters showed minimum toxic effects in the mice which were treated with MGL. After 14 d of transplantation, MGL treated groups were able to reverse the changes in haematological parameters consequent to tumour inoculation.

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