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ANTI TUMOUR ACTIVITY OF *GURU PATHANGAM* AGAINST DALTON ASCITES LYMPHOMA (DAL) IN MICE

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ABSTRACT

The present investigation was taken to explore the anticancer property of the *Guru Pathangam* in swiss albino mice against Dalton ascites lymphoma (DAL). The experimental parameters used were mean survival time, increase in life span, cell count and hematological parameters. The groups were divided in four controls, standard (5-flurouracil) and *Guru Pathangam* in 5 mg/kg for 3 days and 14 days peroral administration. The drugs were administered orally for 14 days to DAL groups except the control group. The siddha formulation produced the significant action against the cancer group's animal in all parameters. The *Guru Pathangam* was found to be good anticancer drug in siddha system.

KEYWORDS: DAL, *Guru Pathangam*, hematological, Mean survival time, Increase life span.

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INTRODUCTION

The traditional system of medicine became significantly more popular all over the globe because of the curative property, less toxic and has no side effects.[1] Siddha system of medicine (SSM) is one such ancient traditional system of India. This interest has led to the discovery of almost lines 8,500 marine natural products to date and many of the compounds have shown very promising biological activity. [2, 3] Indian system of medicine has been widely used for thousands of years in India. Now a day's acceptance of traditional system of medicine in development world is sharply increasing.[4-6] In Siddha system of medicine the drug sources are obtained from plant, mineral, metal and animals.[7] several others have also worked on the efficacy and safety aspects of mercurial preparations in such traditional drugs.[8, 9]

Cancer is a group of more than 100 different diseases. Cancer occurs when cells become abnormal and keep dividing and forming cells without control or order. This uncontrolled cell division and growth ultimately results in cancer. Normally cells divide to produce more cells only when the body needs them. If cells divide when new ones are not needed they form a mass of excess tissue, called tumor. Tumors can benign (not cancer) or malignant (cancer). These three are the characters that differentiate malignant neoplasms from benign tumors that they do not divide abnormally beyond normal limits, do not invade or metastasize. [10]Cancer chemotherapy strives to cause a lethal cytotoxic lesion that can arrest a tumor progression. The chemotherapeutic agents though effective against various types of tumor are not totally free from side effects. [11] Chemotherapy is indicating when neoplasm's are disseminated and not amenable to surgery. Chemotherapy is also used as a supplement to surgery and radiation treatment to attack micro metastases. Usually, in cancer chemotherapy, the major problems that are being encountered are of myelo suppression and anaemia.[12, 13]

Since most of the antineoplastic agents are mutagens, neoplasms may arise ten or more years after the original cancer was cured. Treatment induced neoplasm's are especially a problem after therapy with chemotherapeutic agents. Therefore, the present study focused on evaluation of the anti cancer activity of the siddha medicine "*Guru Pathangam*" against Dalton's ascites lymphoma in mice.

MATERIALS AND METHODS

(i) Animals

The albino mice weighing about 25-35 gm were used. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20-24°C) and light (12 h light: 12 h dark cycle). Animals were kept in polycarbonate cages with laced steel roofs. The animals were acclimatized for one week under laboratory conditions. The study was conducted at the Vel's University, Chennai after obtaining Institutional Animals Ethical Committee clearance bearing the number (XII/ VELS/ COL/10/ CPCSEA/ IAEC/ 23.09.11).

(ii) Drugs and Chemicals

The *Guru Pathangam* prepared traditionally by me at government siddha medical college, Department of PG Gunapadam, Chennai and palm jaggery purchased from Arumbakkam, Chennai. And 5-Flurouracil generously gifted by Orchid chemicals, Chennai and all the other drugs and chemicals used in this study were analytical grade and purchased from sigma chemicals, St. Louis, MO, USA and Qualigens fine chemicals.

(iii) Stock Solution Preparation

The powdered form of *Guru Pathangam* was filtered through cheese cloth and was mixed uniformly in the adjuvant palm jaggery and diluted with saline to achieve 100 mg/ml as main stock solution and used in this study.

(iv) Acute Toxicity Study

The acute oral toxicity study was carried out as per the OECD guidelines-423. Animals were observed individually after administration of *Guru Pathangam* during the first 30 minutes, and periodically 24 hours with special attention during the first 4 hours and daily thereafter for a total of 14 days for toxic symptoms and mortality. All observations were systematically recorded with individual records being maintained for each animal. One-tenth of the lethal dose was considered as therapeutic dose for further pharmacological study. An acute toxicity study was carried out using mice and up to 200 mg/kg dose level. Toxic signs were observed at all the higher dose levels (Up to 50 mg/kg p.o). So, 1/10th of this dose (5 mg/kg) was considered as therapeutic dose for this study. [14]

(v) Cell lines

Dalton ascites lymphoma (DAL) cells were obtained from Amala cancer research institute. Thrissur, Kerala and maintained by weekly intraperitoneal inoculation of 2×10^6 cells/Mouse.

(vi) Groups of the Animals

Group-I Normal control; Group-II Cancer control, DAL cell line (2×10^6 cell mouse); Group-III DAL cell line (2×10^6 cells) treated with 5 mg/kg p.o. of Guru pathangam for 3 days only; Group-IV DAL cell line (2×10^6 cells) treated with 5 mg/kg p.o. of Guru pathangam; Group V-DAL cell line (2×10^6 cells) treated with standard [5-Flurouracil (20 mg/kg i.p)]

(vii) Experimental Procedure

After 14 days of treatment, animals from each group were sacrificed by retro orbital plexus method to evaluate the antitumour potential of *Guru Pathangam*. [15] Group I served as Normal control in which no cancer was induced and allowed to take normal food and water and was treated with 0.9% Sodium chloride only. Group II animals were served as Cancer control induced with DAL cell line (2×10^6 cell mouse) and no active drug was administered and left untreated till death. The Group III animals induced with DAL cell line (2×10^6 cell mouse) and received *Guru Pathangam* treatment for three consecutive days to correlate with clinical study. The Group IV animals induced with DAL cell line (2×10^6 cell mouse) and received *Guru Pathangam* treatment for fourteen consecutive days. Group V was considered as standard treated with 5-FU (20 mg/kg/day i.p). (Dabur Pharmaceutical Ltd, India).

Peritoneal cells were counted 24 hours and last day after drug administration for each of the treated group and compared with those of the untreated group. All treatments were continued for 14 days and median survival times for each group were noted. The animals surviving more than 20 days were considered as cured. The antitumor efficiency of *Guru Pathangam* (5 mg/kg/day p.o. for 14 days) was compared with that of 5-FU. MST was noted with reference control. Survival time of drug treated groups was compared with those of control group. Mean survival time and increased life span (% ILS) was calculated using the following equation. [16-18]

$$\text{MST} = (\text{Day of first death} + \text{day of last death})/2$$

$$\text{ILS} = \frac{\text{MST of treated group}}{\text{MST of the control group}} \times 100 - 100$$

(viii) Hematological Study

In order to determine the hematological status of DAL bearing mice on day 14 after transplantation, the comparison were made amongst normal, tumor bearing mice and tumor bearing mice treated with 5 mg/kg p.o. of *Guru Pathangam*. Blood was drawn from each mouse through retro orbital vein using heparinized capillary tube and the WBC Count, RBC Count, Hb level, Protein and PCV were determined. The ascetic fluids were collected on 14^{th} day and smeared. The smear was stained with Giemsa stain for cytological studies.[19, 20]

(ix) Hemoglobin Concentration of Whole Blood

The concentration of hemoglobin was measured by the usual procedure using Shali's haemometer. Blood sample was drawn into the pipette up to the 20 cu mm mark and transferred to the rectangular cell containing a little amount of N/10 HCl placed in haemometer. After 5 minutes, a color comparison was made with standard color prism of haemometer. If the color of the solution was high, distilled water was added to this solution and mixed using a stirrer until a good color match was obtained. The final reading of the solution in the tube was noted. From the cuvette reading, hemoglobin in g/100ml of blood or its percentage was calculated.

(x) Erythrocyte Count

Blood was taken up to 0.5 marks in the RBC pipette and excess blood was wiped off from the tip. The pipette was then filled to 101 marks with RBC diluting fluid. The RBC pipette was horizontally shaken and a drop of resultant mixture was discharged under the cover glass of a Neubauer counting chamber (Neubauer, Fein optic, Germany). Number of erythrocytes in 80 small squares was counted under the light microscope. The number of cells in 1 ml of undiluted blood was calculated using the standard formula:

$$\text{Erythrocyte count per ml} = \frac{N}{80} \times 1 \times 2000 \times 0.02$$

Where N= number of cells in 80 small squares (dilution)

(xi) Determination of Peritoneal Tumor Cell Count

The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in 64 small squares was counted.

(xii) Total Leukocyte Count

Blood was drawn up to 0.5 mark in the WBC pipette, diluted with WBC diluting fluid up to 11 mark and mixed properly. The resultant mixture was charged under the cover slip in the Neubauer chamber and the number of cells in four-corner block (each block is sub divided into 16 squares) was counted. The total leucocytes count per ml of blood was calculated by multiplying the average number of cells in the four blocks by 200.

(xiii) Packed Cell Volume (PCV)

Using a Pasteur pipette, the wintrobe tube was filled with blood, starting at its bottom and withdrawing the pipette as the tube is filled from below upwards. The blood column was brought to the 'O'. Mark air bubbles, if any were removed from the top of the column of blood so that it stands exactly at 'O'. The tube was centrifuged for about 20 minutes at 25.60 rpm. The reading of the packed cells was taken, the tubes again centrifuged for 5 minutes and the reading was noted. Final reading was recorded when three consecutive readings were identical i.e., when the red cells have been fully packed.[21]

(xiv) Statistical Analysis

All the values were expressed as mean ± SEM. The data were statistically analyzed by one-way ANOVA followed by Dunnett's test. The data of hematological parameters were analyzed using ANOVA followed by Tukey multiple comparison test where $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

(i) Anticancer Study

Hippocrates, the great Greek physician (460-370 B.C), who is considered the father of medicine, was thought to be the first person to clearly recognize difference between benign and malignant tumors. His writings include description of cancers involving various body

sites. Generally, any potential anticancer drug is expected to increase the mean survival time and thus increasing life expectancy. There is a tendency for increase in body weight in tumor bearing mice, which is the result of increased formation and collection of ascites fluid. Potential anticancer drugs reduce the body weight by decreasing the formation of ascites and this effect is due to the cytotoxic against malignant cells, which induce ascites. The tendency for cancer cells is to decrease the peritoneal cell count whereas it is increased in normal animals or those treated with anticancer drugs. In malignancy there is always an alteration of various hematological parameters; increase in a few and decrease in others. One of the major criteria of judging good anticancer drugs is that it should be able to prolong the life and decrease the leucocyte count. [22] Reduced volumes of tumor and increased life span also indicated decrease of cell division. [23]

(ii) Mean Survival Time (MST)

Mice transplanted with DAL in this study have MST of 17.42 days, which was increased to 28.64 by three days treatment and 31.33 days by 14 days administration of *Guru Pathangam* treatment respectively at the dose level of 5 mg/kg p.o, in mice. Tumor bearing mice showed an increase in body weight to the extent of 35 gm. The reliable method for judging the value of the anti cancer drug is the extension of lifespan of the animal and disappearance of leukemia cells from blood. The result revealed the anti tumor effect of *Guru Pathangam* against DAL in Swiss albino mice.

Table 1

Effect of Guru Pathangam treatment on the survival of Dalton's ascites lymphoma (DAL) bearing mice

Group and Treatment	Body weight (g)	MST (Days)	%ILS
Group I -(Normal control)	28.6 ±0.14	44.52±0.22	---
Group II -Cancer control (Saline 2ml/kg)	28.1±0.14	17.42±0.17**	---
Group III -GP (5 mg/Kg p.o. for 3days)	25.93±0.14	28.64±0.17**	64.40
Group IV- GP (5mg/Kg p.o.)	24.7±0.16	31.33±0.13**	79.85
Group V- 5-FU (20 mg/Kg i.p.)	22.8±0.17	33.60±0.12**	92.88

MST = mean survival time; ILS = increased life span. The values are represent MEAN ± SEM (n=6) One Way NOVA followed by Dunnett test where the values are significantly ** P<0.01.

(iii) Estimation of viable tumor cell count:

The cells were then stained with Trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were nonviable. These viable and nonviable cells were counted.

Cell count = (No. of cells x Dilution) / (Area x Thickness of liquid film)

Table 2
Effect of Guru Pathangam treatment on viable and non-viable cell count in DAL bearing mice

Group and Treatment	Viable cell count x 10^6 cells/ml	Non-viable cell count x 10^6 cells/ml
Group I -(Normal control)	---	---
Group II -Cancer control (Saline 2ml/kg)	10.21±0.10	3.68±0.18
Group III -GP (5 mg/kg p.o. for 3days)	3.77±0.05**	1.53±0.07**
Group IV- GP (5mg/kg p.o.)	2.42±0.07**	2.11±0.15**
Group V- 5-FU (20 mg/kg i.p.)	1.06±0.02**	2.98±0.10*

The values are represent MEAN ± SEM (n=6) One Way ANOVA followed by Dunnett test where the values are significantly ** P<0.01.

(iv) Peritoneal Cell Count

Guru Pathangam increases peritoneal cell count in normal mice for three days. Increase in peritoneal cell count induced by *Guru Pathangam* is an indirect indication of their anticancer property. The peritoneal cell count was calculated in individual as 8.2 x 1.4 x 10^6 and enhanced the count as 10.6 ± 1.9 x 10^6 in combinational treatment respectively. The peritoneal ascitic fluid smear result is substantial to the related parameters.

(v) Hematological Parameters

Hematological parameters of tumor bearing mice on day 14 were found to be remarkably altered from the normal group. There is a decrease in Hb, RBC and lymphocytes in malignancy accompanied by an increase in WBC especially neutrophils, protein and PCV. These changes are due to iron deficiency or due to hemolytic of myelopathy conditions induced by malignancy. *Guru Pathangam* have very well reverted the above hematological parameters altered by the transplantable tumor of DAL.

Table 3
Effect of Guru Pathangam on hematological parameters of Dalton's ascitic lymphoma (DAL) bearing mice

Group	Treatment	Hemoglobin (%)	RBC (1x10^6 cells/mm^3)	WBC (1x10^3 cells/mm^3)	PCV (ml)
Group I (Normal)	(Saline 2ml/kg)	13.2 ± 1.5	6.4 ± 2.61	9.4 ±1.02	0.2 ± 0.86
Group II Control	(Saline 2ml/kg)	10.3 ± 1.39ns	2.9 ± 2.36 ns	18.0 ± 3.46*	2.7 ±0.80 ns
Group III	GP (5mg/Kg p.o. for 3days)	11.1 ±1.05ns	3.8 ±1.42ns	15.9 ± 0.97ns	1.4 ± 0.24ns
Group IV	GP (5mg/Kg p.o.)	12.6 ±1.74ns	5.7 ±2.57ns	10.5 ±1.51 ns	0.3 ±0.65 ns
Group V	5-FU (20 mg/Kg i.p.)	13.0 ±0.95ns	6.2 ± 3.32ns	11.1±0.88 ns	0.3 ±0.91ns

Values are MEAN ± SEM (n=6) One Way ANOVA followed by Tukey-Kramer's test. Where the ns P>0.05; *P<0.05

In the differential leukocyte count the neutrophil level was increased while the lymphocyte count decreased. At the same period of time, the *Guru Pathangam* treatment could restore all the altered parameters to normal level. *Guru pathangam* treatment in normal mice showed

remarkable increase in potential cell count and hematological study showed drastic changes in all the tumor-induced animals, which were normalized after 14 days treatment of *Guru Pathangam*. The common problems encountered in cancer chemotherapy are myelo suppression and anemia. Anemia occurring in tumor bearing mice is mainly due to reduction in RBC or hemoglobin production, and this may occur either due to iron deficiency or due to hemolytic or other myelopathy conditions.

Viable cell count of the tumor bearing mice was significantly decreased while non-viable cell counts were increased in *Guru Pathangam* treated groups when compared with DAL treated group. Treatment with *Guru Pathangam* brought back the hemoglobin content, RBC and WBC counts to near normal. This indicates that *Guru Pathangam* have a protective effect on the haemopoietic system. Further, analysis of haematological parameters showed minimum toxic effect in mice treated with *Guru Pathangam.* In DAL bearing mice, hematological parameters were reversed to normal by *Guru Pathangam* administration.

Similarly, Cytological studies of ascetic fluid on the 14th day in DAL bearing mice revealed that the tumor cells are large in size showed binucleation. In *Guru Pathangam* 5mg/kg treated animals, showed plasmacytoid feature with varying degree of degeneration and cytoplasmic vacuolation and also showed active mitosis. All these cytological studies indicate the cytotoxic effect of *Guru Pathangam*. In DAL bearing mice, there was a regular and rapid increase in ascetic fluid volume. Ascites fluid is the direct nutritional source for tumor growth; it meets the nutritional requirement of tumor cells. *Guru Pathangam* treatment decreases the volume of solid tumor as well as ascites volume, viable cancer cell count and increased the life span. It may be concluded that *Guru Pathangam* decreases the nutritional fluid volume and thereby arrest the tumor growth and increase the life span.

CONCLUSION

In the present study the *Guru Pathangam* was studied for its antitumour effect against transplantable tumor. A significant improvement of MST and peritoneal cell count were observed in the tumor-induced animals. From these results, it can be concluded that the *Guru Pathangam* at 5 mg/kg dose level posses anti tumor effect against DAL cells probably by activation of macrophages or by some cytokine product release inside the peritoneal cavity. In conclusion, Hematological parameters of tumor bearing mice on day 14 showed significant changes when compared with normal control.

The total WBC count and PCV were found to increase with a reduction in the hemoglobin content of RBC. At the same time interval*, Guru Pathangam* (5 mg/kg per day p.o.) treatment changed this altered parameters to near normal. The anti tumor effect of the *Guru Pathangam* is evident from the increase in lifespan, reduction in solid tumor volume and also the reversal of altered hematological parameter almost equal to normal. All these data confirmed that the *Guru Pathangam* can be used as a potential agent in the area of cancer chemotherapy. The investigations have to be carried out in characterization and the mechanism involving in antitumor and cytotoxic effect. Hence, it can be concluded that the *Guru Pathangam* has the tumoricidal effect comparable with standard drug 5-FU efficacy and thereby maintain normal physiological profile in mice.

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